Vol. XLII No. 2

February, 1953

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

CONTENTS OF THIS ISSUE

The Application of a Citric Acid Buffer in the Extraction of Solanaceous Alkaloids 63
J. A. Witt, V. Jirawongse, and H. W. Youngken, Jr.

The Preparation and Analysis of Quercetin 66
J. Naghski and C. F. Krewson

Clinical Application of Quercetin: Preliminary Report 68
John Q. Griffith, Jr.

The Influence of a Continuous Injection of Digitalis Tincture on Its Lethal Dose in Pigeons 70
D. P. N. Tsao, A. Farah, and H. W. Youngken, Jr.

The Structure of Substances Which Affect the Autonomic Nervous System. I. Arylvinylamines 72
George P. Hager and Kenneth H. Stahl

The Synthesis of Certain Sulfur-35 Labeled Sulfa Drugs 77
P. J. Byrne, Jr., A. A. Alberts, and J. E. Christian

A Preliminary Study of Silicone Oils as Dermatological Vehicles 79
Joy Bickmore Plein and Elmer M. Plein

The Synthesis of Carbon-14 Labeled Chloroform 85
Henry C. Reitz, Frederick J. Carleton, and John E. Christian

The Preparation of Belladonna and Stramonium Tinctures through the Use of the Colloid Mill 88
S. J. Dean, Donald C. Brodie, E. Brochmann-Hanssen, and Sidney Riegelman

A Study of Suppository Bases. I. Review of Literature 90
Herbert Micheal Gross and Charles H. Becker

A Study of Suppository Bases. II. A Colorimetric Method for Measuring Medicinal Release from Suppository Bases 96
Herbert Micheal Gross and Charles H. Becker

A Method of Evaluating Local Anesthetics 101
Walter L. Mack and John W. Nelson

A New Technique for the Evaluation of Ointment Bases 103
Walter L. Mack, John W. Nelson, and Earl P. Guth

A Study of the Iron-Gentisate Reaction and Its Application 104
John G. Wagner

A Pharmacological Study of Certain Alkoxyalkylamines 107
Tom S. Miya and L. D. Edwards

Pharmacognostical Study of American Berberis 111
V. H. Simonian and H. W. Youngken, Sr.

Examination of the Seed of *Pachyrrhizus erosus* 117
Bella V. Baker and E. V. Lynn

A Note on the Isolation of Furan-3-Carbonic Acid from the Seeds of *Euonymus europaea* L. 119
Egil Ramstad

Book Notices 120

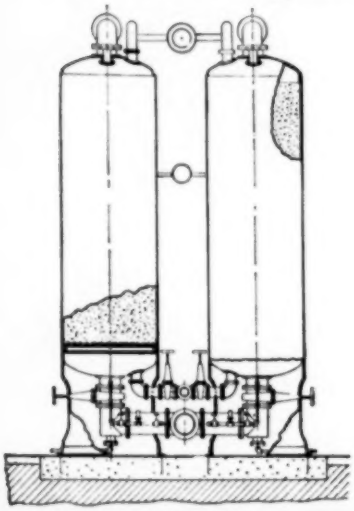
Advertising ..... Cover Pages 2, 3, 4 and pp I–IV

Copyright 1953, by the American Pharmaceutical Association

In the Laboratory ...in the Plant

Florisil

is a highly selective adsorbent . . .



On a scale that was not usual heretofore, quantitative adsorption is now a recognized procedure.

FLORISIL, a high-purity synthetic adsorbent, is now available in quantity for chromatographic separation. An enlarged production unit has just been completed to supply the increased demand for Florisil in new analytical and plant processing procedures. The same controlled quality, uniformity, and purity have been maintained as was formerly expected in our analytical adsorption reagent.

A hard, porous, white granular substance, produced in a variety of mesh classifications, Florisil offers new possibilities in difficult separations of complex organic compounds. Chromatographic adsorption, vitamin analysis, and pharmaceutical purification may be named among approved uses.

Florisil is highly adaptable for pharmaceutical and chemical requirements in both research and manufacture. A new booklet of information has just been released.

FLORIDIN COMPANY

ADSORBENTS
DESICCANTS
DILUENTS

Dept. U 220 Liberty Street Warren, Penna.

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

SCIENTIFIC EDITION

Committee on Publications

B. V. Christensen, *Chairman*
Walter M. Chase
Robert P. Fischelis
Don E. Francke
Hugo H. Schaefer

Editorial Advisory Board

Walter C. Gakenheimer
John E. Christian
George P. Hager
L. W. Hazleton
Elmer B. Vliet
H. W. Youngken, Jr.

JUSTIN L. POWERS, EDITOR

HELEN REYNOLDS, EDITORIAL ASSISTANT

NOTICE TO AUTHORS OF PAPERS

Manuscripts.—All manuscripts submitted to the Editor or presented to the Sections of the ASSOCIATION should be ready for publication, typewritten with double spacing on one side of good quality paper 8.5″ x 11″ in size and with 1″ to 1.5″ margins. In addition to the original typewritten copy, one or more carbon copies should be submitted in order to facilitate examination by members of the Editorial Advisory Board or other referees. Each manuscript should be accompanied by an abstract of not more than 200 words which includes a summarization of conclusions and recommendations. All pages in manuscripts, including tables but not figures, should be numbered consecutively. Previously published and readily accessible procedures which have been followed in experimental work should not be made a part of the manuscript, but should only be referred to by appropriate literature references.

Form of Presentation.—Titles of manuscripts should be kept as short as possible and preferably restricted to one line. The name of the laboratory in which the work described was done should be placed in a footnote at the end of the first page. Each manuscript should begin with one or more introductory paragraphs indicating the intention and meaning of the investigation and the method of procedure. The introduction should be followed by a report of the essential details of the experimental work, and a discussion or interpretation where necessary. Finally there should be a brief summary of conclusions and recommendations. Authors may find it helpful to arrange manuscripts to conform to the style of presentation as illustrated in papers on analogous subjects published in recent issues of THIS JOURNAL.

In preparing manuscripts for publication in THIS JOURNAL, center headings should be avoided as far as possible. Side headings followed by a period and dash should be indented and run into the part of the text to which they apply. Such side headings may be used as freely as desired. Literature citations should be numbered consecutively in order of appearance in the manuscript and should be indicated by full-sized Arabic numerals enclosed in parentheses. If a literature reference is cited more than once in a manuscript, subsequent citations should be indicated by the first number assigned. References to footnotes throughout the text should be numbered consecutively by superscript Arabic numerals, but such references in each table should be designated by superscript lower case letters beginning with "a" and continuing in natural order.

Published by the American Pharmaceutical Association under supervision of the Committee on Publications with the approval of the council. Publication office, 20th and Northampton Sts., Easton, Pa. Editorial office: Scientific Edition, 2215 Constitution Ave., N. W., Washington 7, D. C.

Annual Subscription—Journal of the American Pharmaceutical Association, complete (both editions): United States and Pan America, $7.00; Canada, $7.70; other foreign, $8.00; members of the American Pharmaceutical Association with dues, $4.00. Each edition, Scientific Edition or Practical Pharmacy Edition: United States and Pan America, $4.00; Canada, $4.35; other foreign, $4.50. Single numbers, either edition: United States and Pan America, $0.35; Canada, $0.40; other foreign, $0.50.

Claims for missing numbers will not be allowed (1) if received more than 60 days from date of issue, (2) if loss was due to failure to give 30 days notice of change of address, or (3) if reason for claim is "missing from files."

Entered as second-class matter January 23, 1917, at the Post Office at Easton, Pa., under the act of March 3, 1879, as 24 times a year; Scientific Edition monthly on the fifth; Practical Pharmacy Edition monthly on the 20th. Acceptance for mailing at a special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 10, 1918.

Literature citations should be grouped at the end of the manuscript under the heading "References." All bibliographic references should be checked with the original publications. The citations should be numbered consecutively with full-sized Arabic numerals enclosed in parentheses corresponding to the numbers used in the text. The names of all periodicals cited in the list of references must be abbreviated in accordance with abbreviations given by **Chemical Abstracts** in its "List of Periodicals Abstracted." The sequence followed in the citations must be as follows:

(1) Doe, J. B., *Am. J. Physiol.*, **79**, 289(1927).

References to books should be in the order given, (1) author's surname and initials, (2) title of the book, (3) volume, (4) edition, (5) name of publisher, (6) place of publication, (7) year of publication, and (8) page number. A complete book reference may be illustrated as follows:

(1) Gilman, H., "Organic Chemistry," Vol. 2, 2nd ed., John Wiley and Sons, New York, 1943, pp. 1236, 1252.

Arabic numerals must be used to designate all definite weights, measurements, percentages, and degrees of temperature unless occurring at the beginning of a sentence, in which case the numeral and measurement are spelled out. Periods of time should be written in words. Unusual characters and Greek letters should be written plainly and legibly or should be explained by notes on the margin of the page. Fractions should be written with a slant line. Standard abbreviations should be used whenever weights and measures are stated in the metric system, e. g., 10 Gm., 2.5 cc., etc. The forms to be used are: cc., Kg., Gm., mg., mm., cm., and L.

Figures.—All drawings should be made in India ink, preferably on white tracing paper or cloth. Original drawings are preferable, but clear, well-prepared glossy photographic prints are acceptable. The size of illustrations should not exceed the 8.5″ x 11″ manuscript size. They should be designed for reduction to the width of one column (2.75″) or full page width (5.5″). Thus, height rather than width should be accentuated where possible.

Numbers and measurements, etc., which are to appear on the axes of the drawing should be indicated in pencil outside the axes, in order that they may be set in type. Captions and legends should be placed *below* the diagram (not on it), or, if lengthy, collected on a separate typewritten list, identifying each by its proper figure number and including whatever acknowledgments are necessary.

Photographs should never be rolled, and paper clips should not be used unless guarded by a piece of paper folded several times to prevent an indentation in the photograph. All drawings and photographs should be protected from bending and from the chance of having their edges torn while in transit. Do not write on the back of a photograph. Any indentation in the photograph, however slight, will be likely to show as a blemish in the reproduction. All figures submitted must be referred to in the text of the manuscript, and should be numbered consecutively with Arabic numerals, e. g., Fig. 1, Fig. 2, etc. Kymograph tracings should not be submitted with the original experimental legends which are likely to have been hastily or carelessly inscribed. Authors may find it worth while to employ a professional draftsman to make the drawings in proper form for reproduction.

Tables.—Tables should be carefully constructed so that the data presented may be understood easily. The indiscriminate use of tables should be avoided. Tables should be constructed so as to occupy fully a single column (2.75″) which will take about 40–50 characters and spaces. Tables which require two columns are also acceptable. Interpretative statements should not be written on the body of a table but should be explained in footnotes. Column heads should be made as small as possible and standard abbreviations should be used liberally. Data which may easily be deduced by simple arithmetic from data in another column should not be included. All tables should be referred to in the text of the manuscript. Every table and every column should be supplied with an appropriate heading. It is not necessary to place tables on separate sheets, and in fact it is preferable to intersperse them in the manuscript. Tables should be numbered consecutively using Roman numerals. The table number and the title should be placed in a continuous heading above the data presented. The preparation of a clear and concise table of data requires much planning and infinite care.

The Massachusetts College of Pharmacy

Longwood Avenue, Boston

Graduate study in Boston has always appealed strongly to students in the field of medicine and public health. The environment is stimulating and attracts students from all parts of the world. Library, laboratory, hospital, and other facilities for graduate work are outstanding in this area.

The Massachusetts College of Pharmacy is located in a great medical and educational center. It offers unusual opportunities to graduates of colleges of pharmacy who are interested in further study in pharmacy, pharmaceutical chemistry, and pharmacognosy. A few fellowships are available.

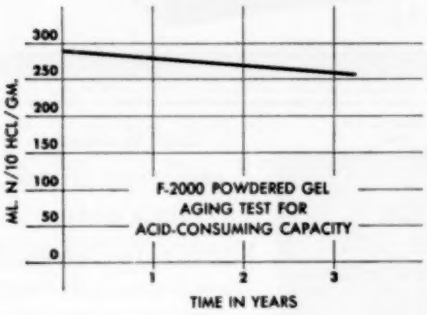
For additional information, write to HOWARD C. NEWTON, Dean

REHEIS

$Al(OH)_3$

DRIED GELS ARE EFFECTIVE LONGER...

3-year aging test proves only slight loss in acid-consuming capacity*



ACTUAL MEASURED VALUES:

At start of test	286
After 1 year	279
After 2 years	270
After 3 years	260

Another reason why Reheis is the standard of quality in dried and compressed gels.

Write for data and samples.

FINE REHEIS CHEMICALS

*Conducted according to U.S.P. XIV acid-consuming capacity test procedure.

8726

REHEIS COMPANY, INC.
Manufacturers of Fine Chemicals
BERKELEY HEIGHTS • NEW JERSEY

Handsome Durable Binders for Journals of the American Pharmaceutical Association

Combination Sets for Practical Pharmacy and Scientific Editions $5.50 a pair
For Single Editions $3.25 each

These binders open flat as a bound book. Made of durable imitation leather, they will preserve your journals permanently. Each cover holds 12 issues (one volume)....Do your own binding. Instructions easy to follow. Mail coupon for binders on 10 day trial.

.....**Mail Coupon Today**.....

SUCKERT LOOSE-LEAF COVER CO.
234 W. LARNED ST. DETROIT 26, MICH.

Mail postpaid....binders for the Journal of the American Pharmaceutical Association ☐ Practical Pharmacy Edition ☐ Scientific Edition for years.....
....Will remit in 10 days or return binders collect.

Name.....

Address.....

.....State..City.....

CENTENNIAL CONVENTION SOUVENIRS

A limited number of the following items are still available:

Souvenir Programs Containing Historical Data and Descriptive Matter **$1.00 each**

Mortars and Pestles Decorated with the Centennial Insignia and Drawing of the Headquarters Building **$3.00 each**

Order from the
American Pharmaceutical Association
2215 Constitution Ave., N.W., Washington 7, D.C.

BOUND VOLUMES

Journal of the American Pharmaceutical Association

$10.00

Order from the Association

2215 Constitution Avenue, N. W.
Washington 7, D. C.

CHOLESTEROL IN ITS MOST ACTIVE AND EFFICIENT FORM

the

AMERCHOLS

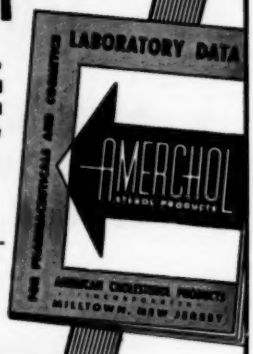
are naturally superior

The AMERCHOLS are surfactants which are NATURALLY good for the skin and hair. They are odorless, color free forms of cholesterol and related rare sterols which we isolate in purified form from animal tissues. They are stable in the presence of acids, alkalies and most drugs.

WE KNOW OF NO CASE OF ALLERGY DUE TO AN AMERCHOL

PHARMACEUTICAL MANUFACTURERS AND CHEMISTS:

Our research laboratories are available to you for advice and information. Our booklet will help you formulate the AMERCHOLS in O/W and W/O emulsions.



AMERICAN CHOLESTEROL PRODUCTS INCORPORATED

MILLTOWN · · · NEW JERSEY

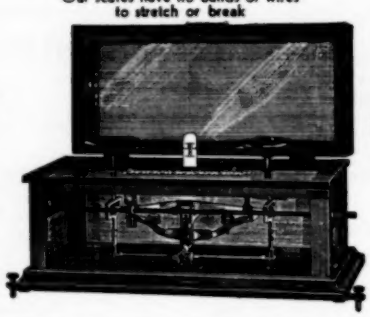
Scale illustrated is our No. 540 all Glass Model

Capacity—4 oz. (120 grams)
Sensitivity—1/10 grain
Finish—Genuine light mahogany with chrome plated parts
Price—$135.00 (subject to change without notice)

PROMPT DELIVERY
on this as well as other class A scales

No. W35 (all glass in lid) $100.00
No. W33 ..... 90.00
All With Stainless Steel Pans

Our scales have no bands or wires to stretch or break



Still the best and lowest priced scale made

HENRY TROEMNER

Manufacturer of the Finest Scales for the Druggist Since 1840

911 Arch Street
Philadelphia 7, Pa.
(Send for Circular A-A)

UNPARALLELED OPPORTUNITY

Offered to right individual who is capable of establishing and growing with his own organization. Objective: development of promotion and distribution facilities for recently developed Swiss therapeutic preparations. Efficacy of preparations and favorable clinical results have been reported in reputable European medical journals. Now in production by Canadian branch. Individual must be experienced in problems of ethical promotion and sales, and be familiar with all aspects of advertising. Preference will be given to person of good character who presently has similar responsibilities, but desires self-employment, and has confidence of medical profession. Communications treated confidentially. **Box 253, Scientific Edition, Journal of the American Pharmaceutical Association, 2215 Constitution Ave., N. W., Washington 7, D. C.**

COME TO PHILADELPHIA FOR PHARMACY

Young men and women from many portions of the United States and from many foreign countries realize the advantages of studies in Pharmacy leading to B.Sc. degrees at this institution, oldest yet most modern of its kind in the Americas.

Graduate studies lead to M.Sc. and D.Sc. degrees. Schools of Chemistry, Bacteriology and Biology also. Write for free catalog.

PHILADELPHIA COLLEGE OF PHARMACY AND SCIENCE

43rd St., Woodland and Kingsessing Aves., Philadelphia 4, Pa. Founded in 1821.

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

VOLUME XLII | *FEBRUARY, 1953* | NUMBER 2 | CONSECUTIVE NO. 4

The Application of a Citric Acid Buffer in the Extraction of Solanaceous Alkaloids*,†

By J. A. WITT, V. JIRAWONGSE, and H. W. YOUNGKEN, Jr.‡

The use of a buffered weak Krebs cycle acid such as citric acid provides a means for extracting total alkaloids from small samples of belladonna and hyoscyamus leaves. However, significantly lower yields are obtained when compared with official procedures for the separation of total alkaloids from *Datura stramonium*.

SEVERAL methods may be found in the literature for accurate separation and determination of solanaceous alkaloids, particularly from raw drug and galenical materials. In general, official methods make use of the conventional immiscible solvent extraction procedures during which ammonia, alkali, ether, chloroform, and a strong acid, such as sulfuric acid, are employed and total alkaloids are finally measured by titration, using an indicator such as methyl red. Such methods require 10-Gm. or larger aliquots of material in the case of solanaceous drugs. They have not proved satisfactory for smaller sample assays (1 Gm. or less) such as are frequently necessary during alkaloid biosynthesis or similar experiments when individual plants must be carefully analyzed.

Modifications of the official methods which are applicable to smaller sample size (1 Gm.) have been reported (1–5). Allport and Wilson (1) have employed 1-Gm. aliquots of stramonium powder for a chemical solvent method during which the chloroform phase is agitated with a weak acid (acetic acid), and alkaloid determination of the acid aqueous phase is made by the Vitali color test. Roberts and James (3) and Fuller and Gibson (4) have employed chromatographic adsorption techniques for preliminary separation purposes; they have found benzene a more efficient solvent for subsequently separating tropane alkaloids than ether, alcohol, or chloroform. The preliminary use of chloroform or alcohol-ether for alkaloid separation such as with the Allport-Wilson method has the disadvantage of extracting bothersome amounts of plant pigments.

It is generally recognized that solanaceous alkaloids as well as others are present in the cell in combination with certain of its cellular constituents, presumably weak acids, proteins, cell wall components, etc. Among weak acids thought significant during the Krebs carboxylic acid cycle of plant metabolism is citric acid (6, 7). Although the true role of the Krebs cycle acids in the formation and utilization of plant amides to form alkaloids remains obscure, it does suggest that in the presence of such acids, alkaloids could be kept in solution within the cell. It was therefore deemed desirable to ascertain the effectiveness of such a natural plant acid for the initial extraction of alkaloids from cells known to

* Received August 22, 1952, from the College of Pharmacy, University of Washington, Seattle.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.
† Supported in part by a grant from the State of Washington Fund for Medical and Biological Research, University of Washington.
‡ Drug Plant Laboratory, College of Pharmacy, University of Washington.

contain them. This report describes the results when such a method is applied to the preliminary extraction of alkaloids from samples of 1 Gm. or less of solanaceous leaf material.

EXPERIMENTAL

Authentic powdered (No. 60 mesh) leaf drug of official belladonna, hyoscyamus, and stramonium from a commercial source[1] and 500 Gm. of dried powdered *Datura stramonium* leaves from the Drug Plant Gardens, University of Washington, were selected for comparative study. Aliquots of each lot were dried overnight in a desiccator and then made ready for total alkaloid (as atropine) separation.

The Citric Acid Buffer Method.—Each of several 1- and 0.5-Gm. aliquots, of the powdered leaf material was placed into a 250-ml. glass stoppered Erlenmeyer flask with approximately 10 ml. of McIlvaine citric acid buffer (8). Three different quantities (2, 4, and 6 mg.) of atropine base (U. S. P.) were similarly processed. The citric acid-monocitrate McIlvaine buffer system was selected because of its simplicity and universal availability. This buffer was made by mixing proportions of 4.11 Na_2HPO_4 (0.2 *M*) with 15.89 citric acid (0.1 *M*) for a *p*H of 3.0 ± 0.2, and is representative of the lower acid range of the McIlvaine table. It seemed reasonable to assume that separation would best take place at a low *p*H range. A buffered solution of citric acid was chosen to keep the *p*H of the acid extraction solution within 0.3 *p*H unit despite extraction of basic water-soluble plant constituents. Plant material was then shaken vigorously in this solution by a mechanical rotary shaker for varying periods of time, i. e., three, four, twelve, twenty-four, forty-eight, and seventy-two hours. A yellowish extract was produced during extraction. However, no appreciable amounts of chlorophyll or other water-insoluble pigments were observed to separate. From alkaloid analyses presently to be discussed, it was found that optimum extraction was accomplished following a shaking period of not less than four hours and not more than twenty-four hours.

The citric acid extract was then filtered by Whatman No. 1 or Eaton No. 615 paper, and the filtrate was collected in 25-ml. volumetric flasks. The marc was washed with approximately 15 ml. of citric acid buffer to make a volume of 25 ml. Thereupon 3-ml. aliquots of the liquid extract were made alkaline with approximately 0.3 ml. of ammonium hydroxide T. S. and then were shaken vigorously in a glass-stoppered flask with 25 ml. of anhydrous benzene C. P. The benzene layer, which was practically colorless at this stage, was allowed to separate. Occasionally the citric acid layer was repeatedly extracted with additional quantities of benzene to insure that complete extraction was effected. In all cases, complete extraction was accomplished with the first benzene shaking. It was therefore concluded that one initial benzene extraction was entirely satisfactory for this procedure.

Each of several 3-ml. aliquots of the colorless benzene extraction solution, removed by pipetting, was then evaporated just to dryness in a No. 000 evaporating dish on a water bath. Care was taken to prevent excessive heating.

The residue was then examined according to the Vitali-Morin method as follows: it was first taken up with 0.2 ml. of fuming nitric acid (C. P.) and then evaporated just to dryness on a water bath. The residue was next dissolved in a few milliliters of acetone (dried over calcium chloride) and placed in a 10-ml. volumetric flask, whereupon the liquid was made up to the 10-ml. mark with more acetone. At this point the acetone solution was thoroughly mixed with 0.1 ml. of a freshly prepared 3% solution of potassium hydroxide in methyl alcohol. When thoroughly mixed, and at exactly five minutes after mixing, the resultant Vitali color produced was measured in a Bausch and Lomb colorimeter with a 550 mμ interference filter. Results were compared with a standard atropine base reference curve. This was previously constructed by similarly measuring the per cent transmission of aliquot portions (0.01, 0.03, 0.05, 0.07, 0.09, and 0.10 mg./ml.) of standardized atropine U. S. P. in anhydrous benzene C. P.

Results of the citric acid buffer-alkaloid extraction method were then compared with alkaloid extractions of 10- or 25-Gm. samples of the same material according to the U. S. P. XIV (9) or N. F. IX (10) methods wherein leaf material was first made alkaline with ammonia T. S., macerated with ether-alcohol overnight, and then extracted with successive chloroform- and acid-immiscible solvents.

RESULTS

Results of the analyses of small amounts of atropine base U. S. P., shaken with 25 ml. of McIlvaine citric acid buffer for twenty-four hours and extracted with benzene C. P., are shown in Table I. It will be observed that the per cent recovery was good, thus indicating satisfactory extraction of the atropine citric acid buffer solution with benzene by this method. Further, this indicated that no apparent hydrolysis of the atropine took place during the twenty-four hour period.

TABLE I.—ANALYSES OF ATROPINE U. S. P. BY THE CITRIC ACID METHOD[a]

Sample No.	Mg. Added	Mg. Recovered[b]	Recovery, %
1	2.02	2.09	103.4
2	2.02	2.10	104.0
3	4.04	3.96	98.0
4	4.04	3.93	97.0
5	6.06	5.88	97.0
6	6.06	5.96	98.3
		Mean average:	99.6

[a] Twenty-four hour shaking.
[b] Average of three determinations.

In Table II are shown comparative results of the assay of hyoscyamus and belladonna leaf drugs by the official method and by the citric acid buffer extractions. There is no significant difference between results found in the case of hyoscyamus. However, the citric acid method gave somewhat higher yields with belladonna leaves. Calculation of the standard deviation indicates that individual yields obtained by the latter method were, however,

[1] From the S. B. Penick Company, 50 Church Street, New York, N. Y.

TABLE II.—COMPARISON OF OFFICIAL AND CITRIC ACID ASSAY METHODS FOR HYOSCYAMUS AND BELLADONNA LEAF

Sample No.	N. F. Method (25 Gm.), Mg./Gm.	Citric Acid (1 Gm.), Mg./Gm.	Sample No.	U. S. P. Method (10 Gm.), Mg./Gm.	Citric Acid Method (0.5 Gm.), Mg./Gm.
	Hyoscyamus			Belladonna	
1	0.44	0.46	1	4.94	5.89
2	0.51	0.50	2	5.05	5.09
3	0.44	0.46	3	4.94	6.03
4	0.37	0.45	4	4.94	5.82
5	..	0.46	5	4.36	5.92
Average:	0.44	0.46	6	4.69	5.85
			Average:	4.82	5.92
S. D.[a] =	±0.06 Mg./Gm.	±0.06 Mg./Gm.	S. D.[a] =	±0.26 Mg./Gm.	±5.95 Mg./Gm.

[a] Standard Deviation calculated as $\sigma = \sqrt{\frac{\Sigma d^2}{N-1}}$

more consistent than those obtained by the corresponding official assay, despite the smaller sample.

Results of the analysis of stramonium powder are shown in Table III. Here it will be seen that consistently lower yields were obtained by the citric acid technique. The fact that 19 determinations gave such results attaches significance to this finding. Furthermore, these determinations were made at widely different periods with the same lot of drug material. Official assay determinations of total alkaloid in representative samples of the marc of several citric acid extractions gave negative results.

The McIlvaine buffer technique in the case of *Datura stramonium* presents a discrepancy which at this time remains unanswered. It raises the question, based on the consistently favorable results with two other solanaceous drugs, of whether the characteristic alkaloids of *Datura* are bound up in cells in a significantly different manner, with respect to citric acid extraction, than are the alkaloids of *Atropa* and *Hyoscyamus* species. Further investigations of this discrepancy are under way.

TABLE III.—COMPARISON OF OFFICIAL AND CITRIC ACID ASSAY WITH DATURA STRAMONIUM LEAF

Sample	U. S. P. (10 Gm.), Mg./Gm.	Citric Acid (1 Gm.) Mg./Gm.
1	3.54	1.59
2	3.51	1.79
3	3.59	1.54
4	3.59	1.85
5	3.48	1.73
6	3.60	1.66
7	2.73	1.69
8	2.90	1.66
9	3.54	1.55
10	3.60	1.53
11	3.32	1.81
12	3.19	1.76
13	3.10	1.62
14	3.57	1.62
15	3.57	1.60
16	3.36	1.54
17	3.19	1.54
18	..	1.60
19	..	1.54
Mean Average:	3.375	1.64
S. D.[a] =	±0.26 Mg./Gm.	±0.10 Mg./Gm.

[a] Standard deviation calculated as $\sigma = \sqrt{\frac{\Sigma d^2}{N-1}}$

SUMMARY

A study has been made of the use of a natural plant acid, such as citric acid, for preliminary separation of total alkaloids from 1-Gm. samples or less of three solanaceous leaf drugs. Results were compared in each case with those obtained by the use of official procedures.

Citric acid, as McIlvaine's citric acid monocitrate buffer, proved an efficient acid for the extraction of alkaloids from small samples of belladonna and hyoscyamus. It was not, however, satisfactory for efficient separation of total alkaloids from *Datura stramonium* under the same conditions.

REFERENCES

(1) Allport, N., and Wilson, E. S., *Quart. J. Pharm. Pharmacol.*, **12**, 399(1939).
(2) James, W. O., and Roberts, M., *ibid.*, **18**, 29(1945).
(3) Roberts, M., and James, W. O., *ibid.*, **20**, 1(1947).
(4) Fuller, W. C., and Gibson, M. R., THIS JOURNAL, **41**, 263(1952).
(5) Dykstra, S. P., *Pharm. Weekblad*, **86**, 129(1951); through *Chem. Abstr.*, **45**, 5876f(1951).
(6) Hoagland, D. R., "Inorganic Plant Nutrition," Chronica Botanica Co., Waltham, Mass., 1948, pp. 135–141.
(7) Dawson, R. F., *Ind. Eng. Chem.*, **44**, 266(1952).
(8) McIlvaine, T. C., *J. Biol. Chem.*, **49**, 183(1921).
(9) "The United States Pharmacopeia," Fourteenth Revision, Mack Publishing Co., Easton, Pa., 1950, p. 72.
(10) "The National Formulary," 9th ed., Mack Publishing Co., Easton, Pa., 1950, pp. 254, 505.

The Preparation and Analysis of Quercetin*

By J. NAGHSKI and C. F. KREWSON†

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) was prepared by hydrolysis with dilute acids of the naturally occurring glycosides, rutin and quercitrin. Data were obtained on the rate of hydrolysis of pure glycosides in concentrations from 2 to 10%.

Our laboratory has had repeated requests for supplies of quercetin for pharmacological and clinical testing. Since pure quercetin is not an article of commerce, methods for its preparation and purification from various sources were investigated.

The occurrence, identification, and chemistry of quercetin have been reviewed by Perkin and Everest (7). Quercetin occurs in many plants in the form of glycosides. Although the hydrolysis of flavonol glycosides to the aglycone is a common step in the characterization of these substances, there is no report in the literature of a systematic investigation of this procedure. The most common method consisted of boiling dilute solutions of the glycosides with 2 to 5 per cent aqueous sulfuric acid for one to two hours (7). Large-scale hydrolyses of glycosides have been developed by dyestuff manufacturers, but the conditions used have been maintained as commercial secrets.

The most abundant glycosides of quercetin are rutin and quercitrin. A simplified procedure for preparing quercitrin from lemon flavine and its hydrolysis to quercetin has been reported by Booth and DeEds (1). However, in view of the possibly limited supplies of lemon flavine, methods for preparing quercetin from the commercially available pure glycoside, rutin, have been investigated and are presented here. Preliminary findings on the clinical application of quercetin in the treatment of capillary fault are presented by Griffith (4) in another report.

EXPERIMENTAL

Since preliminary small-scale experiments had indicated that optimum conditions for the acid hydrolysis of rutin varied, it was decided to study the effect of such factors as the concentrations of rutin and acid, the size of batch, and the time of hydrolysis.

Hydrolysis.—A quantity of rutin sufficient to give the desired concentration was placed in a flask fitted with a side arm for removing samples. The required amount of boiling aqueous sulfuric acid was introduced and boiling was continued under reflux with active stirring.[1] Aliquots were removed at stated intervals, diluted with an equal volume of cold water, and stored in a refrigerator for twelve to sixteen hours. The product was recovered by filtration and washed three times by slurrying with a volume of water equal to the original aliquot and filtering.

Moisture.—The bulk of the water was removed from the cake by drying overnight at 110°. Moisture for analytical purposes was determined by using the same procedure recommended for the drying of rutin (6). Pure quercetin did not appear to be hygroscopic at relative humidities below 50% and so could be dried satisfactorily in a vacuum oven even at 80°. However, the presence of unhydrolyzed rutin will make it necessary to employ the more rigorous conditions recommended for drying rutin in order to obtain the correct moisture value.

Assay.—The ultraviolet spectrophotometric method as developed for the analysis of rutin (8, 9) was extended to the assay of quercetin. The specific extinction coefficients[2] for highly purified rutin (2) and quercetin[3] are presented in Table I. The values shown for quercetin are appreciably higher than those reported in the literature summarized by Booth and DeEds (1). The previous highest value for $K_{375.0}$ was 77.3, reported by Gage, Douglass, and Wender (3).

When the absorbance ratio at 375.0 and 362.5 mμ fell within the limits of 1.108 ± 0.020, the per cent quercetin was calculated directly from the observed absorbance at 375.0 mμ by the equation $Q = 100A_{375.0}/80.0\ lc$. When the absorbance ratio was less than 1.088, the rutin and quercetin were calculated using the simultaneous equations for the determination of these constituents in rutin preparations (9). This two wave-length procedure, however, lacked sensitivity when the relative concentration of quercetin/rutin was greater than 85/15 and so was not satisfactory for the determination

* Received December 1, 1952, from the Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture, Philadelphia, Pa.

Presented to the Scientific Section, A. Ph. A., Philadelphia meeting, August, 1952.

† The authors wish to express their appreciation to B. A. Brice and A. Turner, Jr., for their interest and advice during the course of this study and to Ruth B. Kelly and Mary Anne Morris for analysis of the quercetin preparations.

[1] The solution should boil vigorously; heating on a steam bath is not sufficient to produce complete hydrolysis even with the strong acid. Stirring is essential to keep the quercetin from bumping, especially with the more concentrated slurries of rutin which have a tendency to form a paste at incipient hydrolysis.

[2] The specific extinction coefficient is defined as $K = A/lc$, where A is the absorbance (negative logarithm of the transmittancy), l is the cell length in centimeters, and c is the concentration of solute in grams per liter of solution, in accordance with official nomenclature of the National Formulary IX (9), p. 767.

[3] The highly purified quercetin was prepared by W. L. Porter of this laboratory, according to the following procedure. Refined rutin was hydrolyzed with 0.6% aqueous sulfuric acid and the quercetin obtained was further purified by alternate passage through the pentacetate (5) and the sulfate (5) for a total of five preparations of each derivative. The pentacetate was recrystallized each time from 70% ethanol. The quercetin was recovered after each preparation of the derivatives and analyzed spectrophotometrically. Three passages through the derivatives raised the $K_{375.0}$ to 80.0 which remained unchanged for two additional passages.

of small amounts of rutin in the presence of large amounts of quercetin. Although such inaccuracy would preclude the use of this procedure as an analytical method for the determination of small proportions of rutin in quercetin, the results obtained were useful for comparing the hydrolytic conditions studied. No accurate method is available for such determinations at present.

TABLE I.—SPECIFIC EXTINCTION COEFFICIENTS FOR RUTIN AND QUERCETIN

Wave Length, mμ	Specific Extinction Coefficient	
	Rutin	Quercetin
257.7	37.7[a]	75.7[a]
362.5	32.55[a]	72.2
375.0	28.48	80.0[a]
Absorbance Ratio[b]	0.875 ± 0.004	1.108 ± 0.020

[a] Maxima.
[b] $A_{375.0}/A_{362.5}$ or $K_{375.0}/K_{362.5}$.

DISCUSSION

The rates of hydrolysis of different concentrations of rutin by aqueous solutions of sulfuric acid of varying strength are shown in Fig. 1. The data indicate that rutin is hydrolyzed rapidly during the early part of the reaction, but the curve becomes asymptotic as the reaction approaches completion. Increasing the acid concentration or reducing the rutin concentration increases the rate of hydrolysis.

The composition of several lots of quercetin prepared by the hydrolysis of rutin was determined approximately by the spectrophotometric method. These samples were selected from the study of conditions for large-scale preparation, and the results are presented in Table II. These results indicate that quercetin assaying better than 95% can be obtained by hydrolyzing 2% rutin slurries for one hour with both 2 and 5% solutions of sulfuric acid. More concentrated slurries of rutin require up to four hours of heating to approach the same degree of hydrolysis. Since quercetin is practically insoluble in cold water, yields within 1 or 2% of theoretical were obtained at complete hydrolysis.

Since it was thought that rutin was the main impurity in quercetin prepared by the hydrolysis of rutin, the possibility of improving the purity of quercetin preparations assaying less than 95% of quercetin by extraction with boiling water was investigated. Three such preparations were extracted three times by boiling a 2% slurry with distilled water and filtering while hot. These samples analyzed 91.0, 92.6, and 93.5% quercetin before extraction and 95.0, 95.7, and 96.6% quercetin, respectively, after the hot water extraction. This process increased the quercetin content of the samples only by an amount equal to that which could be produced by an additional one to two hours of heating during the acid hydrolysis. Thus these results indicate that rutin is probably not the only impurity present but that the quercetin preparations contain 3–4% of an unidentified substance. The data in Table II further show that this unknown substance is highly resistant to acid hydrolysis since the quercetin content was not appreciably increased in the one sample which was heated for eight hours.

TABLE II.—HYDROLYSIS OF RUTIN TO QUERCETIN BY BOILING AQUEOUS SULFURIC ACID SOLUTIONS

Conditions of Hydrolysis			Time of Hydrolysis, Hrs.		
Rutin Concn., %	Acid Concn., %	Vol. of Soln., Cc.	1	2	4
			Quercetin Assay, %		
10[a]	5	2,000	93.9[c]	94.9	95.3
10[a]	2	2,000	34.8[d]	83.7	95.1[e]
5[a]	2	2,000	75.6[f]	91.0	96.5
5[a]	1	150	53.1	91.3	95.9[g]
2[b]	5	500	97.6	..	..
2[b]	2	500	96.5	..	..
2[b]	1	500	94.7	..	..
2[b]	0.5	500	77.8	..	..

[a] Rutin was N. F. IX quality.
[b] Rutin purified once by recrystallization as the alcoholate (6).
[c] 53.7% after 1/2 hr.
[d] 16.0% after 1/2 hr.
[e] 92.6% after 3 hr.
[f] 34.4% after 1/2 hr.
[g] 95.6% after 3 hr.; 96.1% after 8 hr.

To determine the feasibility of using crude rutin to make quercetin, samples of the latter were prepared by the hydrolysis of crude buckwheat rutin and crude Sophora rutin. The products obtained were dark colored and contained such excessive amounts of materials which were insoluble in alcohol that they could not be analyzed. Even after one recrystallization from 80% ethanol, the samples were still dark in color and assayed only 93.5%

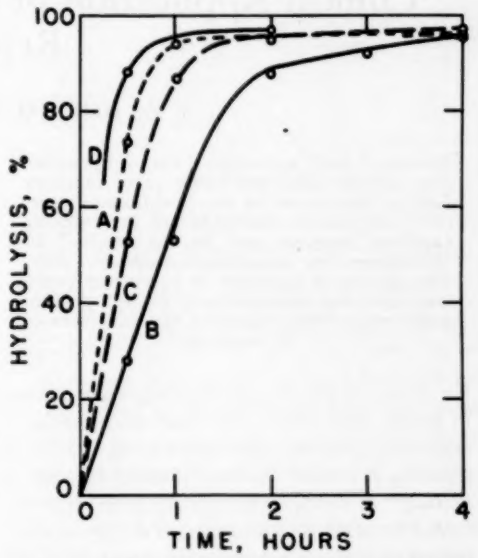


Fig. 1.—Effect of concentrations of rutin, acid, and size of batch on rate of hydrolysis of rutin by boiling aqueous sulfuric acid. *A:* 10% rutin, 5% acid, 2,000 cc. volume; *B:* 10% rutin, 2% acid, 2,000 cc. volume; *C:* 5% rutin, 2% acid, 2,000 cc. volume; *D:* 5% rutin, 2% acid, 150 cc. volume.

quercetin. Furthermore, the quercetin did not recrystallize readily. The crystals were minute and difficult to recover. Thus it was found that the

preparation of quercetin was greatly simplified by starting with purified rutin. The color of the rutin is especially important; a sample that met N. F. IX specifications but was off-color yielded a quercetin which was also off-color.

We have prepared a 3-Kg. batch of quercetin by hydrolyzing a 4.5% slurry of pure quercitrin with 2% sulfuric acid. The hydrolysis was carried out in a 12-L. round-bottom flask heated with an electric heating mantle and with mechanical stirring. The product obtained assayed 97% quercetin (specific extinction coefficient, 77.6) and contained less than 0.01% of ash.

From the data at hand it can be concluded that quercetin assaying more than 95% can be prepared by the acid hydrolysis of the purified glycosides, rutin and quercitrin.

SUMMARY

Quercetin of 95 to 98 per cent purity was prepared from rutin of N. F. IX quality by hydrolysis of aqueous slurries containing 2 to 10 per cent rutin and 1 to 5 per cent sulfuric acid.

Quercitrin can be hydrolyzed under similar conditions.

REFERENCES

(1) Booth, A. N., and DeEds, F., THIS JOURNAL, **40**, 384 (1951).
(2) Couch, J. F., Naghski, J., and Porter, W. L., "Method of Purifying Rutin," U. S. pat. 2,520,127 (August 29, 1950).
(3) Gage, T. B., Douglass, C. D., and Wender, S. H., *Proc. Oklahoma Acad. Sci.*, **29**, 64(1948).
(4) Griffith, J. Q., Jr., THIS JOURNAL, **42**, 68(1953).
(5) Morrow, C. A., and Sandstrom, W. M., "Biochemical Laboratory Methods for Students of the Biological Sciences," John Wiley & Sons, Inc., New York, 1935, p. 295.
(6) Naghski, J., Mellon, E. F., Korn, A. H., and Ogg, C. L., THIS JOURNAL, **41**, 599(1952).
(7) Perkin, A. G., and Everest, A. E., "The Natural Organic Colouring Matters," Longmans, Green and Co., London, 1918.
(8) Porter, W. L., Brice, B. A., Copley, M. J., and Couch, J. F., "Tentative Spectrophotometric Method for the Determination of Rutin in Various Preparations," U. S. Dept. Agr., Bur. Agr. & Ind. Chem. AIC-150 (Eastern Regional Research Laboratory), July, 1947 (Processed).
(9) "The National Formulary," 9th ed., Mack Publishing Co., Easton, Pa., 1950, p. 438.

Clinical Application of Quercetin: Preliminary Report*

By JOHN Q. GRIFFITH, Jr.†

A clinical study was carried out on hypertensive subjects who had evidence of capillary fault as determined by the Göthlin and negative overpressure methods for determining capillary fragility and by the method of McMasters for cutaneous lymphatic flow. The efficacy of quercetin in correcting capillary fault was demonstrated in this preliminary series which involved the treatment of 65 subjects.

IN 1944, Griffith, Couch, and Lindauer (7) reported that rutin (the rhamno-glucoside of quercetin) possessed the physiological activity of restoring increased capillary fragility in man to normal. Since then, these results have been confirmed by other workers, some of whom have extended the application to the treatment of related vascular abnormalities (3–5, 13–16). A more extensive review of the clinical application is presented by Couch, Krewson, and Naghski (4) and by Paris (12).

In subsequent reports, Griffith and Lindauer (8, 10) found the incidence of increased capillary fragility in the hypertensives studied to be about 18 per cent. In a more recent study (6), as yet unreported, the term "capillary fault" was introduced to refer to abnormalities in either fragility or permeability. In the 1,540 subjects studied, increased capillary fragility occurred in 248 (16 per cent) and increased capillary permeability in 31 (2 per cent). Also, the results of rutin therapy have been evaluated in comparison with morbidity and mortality statistics. It was found that when capillary fault (associated with hypertension) was corrected by rutin, the incidence of apoplexy was reduced by 90 per cent and that of coronary occlusion by 60–65 per cent. It was also observed that rutin failed to cause any change in capillary fault in approximately 6–12 per cent of cases even when therapy was followed conscientiously by the patients.

The pharmacology of rutin has recently been reviewed (4, 12) and it appears that its physio-

* Received December 1, 1952, from the Research Foundation, 133 South 36th Street, Philadelphia, Pa.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.
† The author wishes to thank Dr. C. F. Krewson and Dr. J. Naghski of the Eastern Regional Research Laboratory, Philadelphia, Pa., for the quercetin and for their cooperation in these studies; and the Abbott Laboratories, North Chicago, Ill., for capsulating the quercetin.

logical activity may reside in the flavonol moiety (quercetin). It has recently been demonstrated by Ambrose, Robbins, and DeEds (1) that quercetin is nontoxic. Although numerous studies have been carried out with animals, none of these have indicated whether quercetin would be effective for the correction of spontaneous capillary fault in humans. The present study was undertaken to evaluate the clinical efficacy of this compound; results obtained with the first 65 subjects treated with quercetin are presented in this paper. These studies are being expanded in order to obtain sufficient data for a statistical evaluation. The preparation and analysis of quercetin is described by Naghski and Krewson in an accompanying paper (11).

EXPERIMENTAL

Procedure.—Capillary fault in hypertensive patients was determined by a previously described modification (8) of the method originated by Göthlin and also by a negative overpressure procedure (2). Cutaneous lymphatic flow was measured by determining the rate of spread of a dye, patent blue, injected in minute amounts into the skin (9). An abnormal value obtained with one or more of the three tests was considered evidence of capillary fault. The quercetin dosage prescribed was 30 mg./day. However, if capillary fault had not returned completely to normal on a repeat test after a three-month interval, the dosage of quercetin was increased by 10 mg. With several subjects the dosage had to be increased to as high as 50 mg./day.

DISCUSSION

This series included 65 hypertensive subjects who had evidence of capillary fault, indicated by an abnormal reaction to one or more of the tests. These individuals have been taking quercetin from three to eighteen months without any toxic manifestations. The results of treatment with quercetin as determined by repeated tests is presented in Table I. The subjects with capillary fault are classified into three categories, depending on the results of previous therapy with rutin. The first group consists of 32 individuals who were seen for the first time and had no previous therapy. Results of follow-up tests indicated that quercetin was effective in correcting capillary fault in 31 (97%) of these cases. The one subject who did not respond had not as yet received increased dosage. The second group consisted of 23 individuals who were receiving rutin (60–350 mg. daily) but whose capillary fault was not corrected by rutin therapy. Results of follow-up tests indicated that quercetin was effective in correcting capillary fault in 22 (96%) of these cases. The third group consisted of 10 individuals who had previously received rutin and whose capillary fault was corrected by the rutin therapy. In this third group, followed for three to eighteen months, the tests remained normal when they were transferred to quercetin therapy. Previous experience has shown that patients who stop rutin therapy have a recurrence of capillary fault.

From the results of this study it seems evident that quercetin is effective in correcting capillary fault associated with hypertension and appears to be indicated for the treatment of cases that do not respond to rutin therapy.

SUMMARY

In a preliminary clinical study, quercetin was found to correct capillary fault associated with hypertension in 31 of 32 subjects who had not been previously treated with flavonoids. (The one case not responding will receive an increased dosage.)

Ten subjects whose capillary fault was corrected by rutin were transferred to quercetin without suffering a relapse.

Quercetin was found effective in correcting capillary fault in 22 of 23 subjects who did not respond to rutin therapy.

TABLE I.—EFFECT OF QUERCETIN THERAPY ON CAPILLARY FAULT ASSOCIATED WITH HYPERTENSION

No. of Subjects	Previous Treatment	No. of Subjects with —Capillary Fault— Before Quercetin	After Quercetin
32	None	32	1
23	Rutin	23	1
10	Rutin	0	0

REFERENCES

(1) Ambrose, A. M., Robbins, D. J., and DeEds, F., THIS JOURNAL, **41**, 119(1952).
(2) Brown, E. E., *J. Lab. Clin. Med.*, **34**, 1714(1949).
(3) Burger, H., *Z. Geburtshülfe u. Gynäkol.*, **135** (2), 182–193 (1951).
(4) Couch, J. F., Krewson, C. F., and Naghski, J., "Chemistry, Pharmacology, and Clinical Applications of Rutin," U. S. Dept. Agr., Bur. Agr. & Ind. Chem. AIC-291 (Eastern Regional Research Laboratory), February, 1951 (Processed); also *Bull. Natl. Formulary Comm.*, **19**, 46 (1951).
(5) Donegan, J. M., and Thomas, W. A., *Am. J. Ophthalmology*, **31**, 671(1948).
(6) Griffith, J. Q., Jr., and Couch, J. F., "Rutin" (monograph, in preparation).
(7) Griffith, J. Q., Jr., Couch, J. F., and Lindauer, M. A., *Proc. Soc. Exptl. Biol. Med.*, **55**, 228(1944).
(8) Griffith, J. Q., Jr., and Lindauer, M. A., *Am. Heart J.*, **28**, 758(1944).
(9) Griffith, J. Q., Jr., Roberts, E., Rutherford, R. B., and Corbit, H. O., *ibid.*, **21**, 62(1941).
(10) Griffith, J. Q., Jr., *Inst. Med. Chicago Proc.*, **16**, No. 16 (Oct. 15, 1949).
(11) Naghski, J., and Krewson, C. F., THIS JOURNAL, **42**, 66(1953).
(12) Paris, R., *Produits pharm.*, **6** (11 and 12), 543, 606 (1951).
(13) Von Schmidt, H., Marx, R., and Festl, B., *Deut. med. Wochschr.*, **75** (23), 790(1950).
(14) Schweirer, P., *Klin. Wochschr.*, **29**, 328(1951).
(15) Shanno, R. L., *Am. J. Med. Sci.*, **211**, 539(1946).
(16) Zfass, H. S., *Virginia Med. Monthly*, **75**, 56(1947).

The Influence of a Continuous Injection of Digitalis Tincture on Its Lethal Dose in Pigeons*,†

By D. P. N. TSAO,‡ A. FARAH,§ and H. W. YOUNGKEN, Jr.[1]

A study of a wide dose range of digitalis tincture injected into pigeons by a continuous injection method indicates that with this method there is no significant change in the lethal dose at levels of from 0.0025 to 0.0226 cc./Kg./min. during an experimental period of from forty-two to 340 minutes.

STUDIES of the influence of the rate of administration on the lethal dose of digitalis tincture and the isolated cardiotonic glycosides when studied in intact cats has produced somewhat variable results (1–5). Bliss and Allmark (3) reported that with a twofold increase in the amount of tincture, the lethal dose increased by 15 per cent. Matuda (4) showed that with lower rates of administration over a longer time increment, significantly higher lethal doses could be expected. Haag and Woodley (6) state that rates slower than 0.5 cc. per ten-minute intervals in pigeons offer no advantage but might provide for appreciable elimination, thus affecting the dose. Maresch and Farah (5), using cats, showed that with high rates of continuous injection of digitoxin and other glycosides the lethal dose was high, but that with decreasing rates the lethal dose fell until a kind of plateau could be reached at a very wide range of slow injections. The dose at such a time has been referred to by these authors and others as the minimum lethal dose.

The object of this study has been to examine the effect of several slow rates of a continuous injection of digitalis tincture on the lethal dose in pigeons and to compare the experimental time from such dosage.

MATERIALS AND METHODS

Digitalis tincture was prepared according to the following method: a suitable amount of U. S. P. Digitalis Reference Standard power which had been put into a desiccator over sulfuric acid for twenty-four hours was weighed and transferred immediately into a glass-stoppered Erlenmeyer flask. A sufficient menstruum consisting of 4 parts of alcohol and 1 part of distilled water by volume was added so that the total volume of menstruum added corresponded to 10 cc. of menstruum for each gram of powder. The stopper was inserted immediately in order to prevent any evaporation. The upper third of the stopper was greased lightly with petrolatum. The mixture was shaken for twenty-four hours at room temperature by an electric shaking machine. Thereafter, the mixture was immediately centrifuged and filtered into a dry neutral glass ampul and the contents were well mixed. During the filtration the funnel was covered to avoid any evaporation. Pure nitrogen was passed into the ampul containing the tincture for five minutes to displace the air. The rubber cap was immediately inserted and the ampul was then stored under refrigeration until time for its use.

The U. S. P. XIV biological procedure for digitalis tincture (7) was modified by providing a continuous injection into the alar vein of the pigeon instead of the five-minute interval injection procedure. Pentobarbital sodium (40 mg./Kg.) was used in place of anesthetic ether to provide a more convenient anesthesia. The continuous injection modification was found to be more convenient than the intermittent (five-minute) injection technique of the U. S. P. and possessed the advantage of decreasing the incidence of blood clotting in the vein. It was carried out by the use of a Kniazuk Merck Laboratory Constant Rate Infusion Pump[1] into which a 20-cc. standardized syringe was inserted. Fluid was forced through the syringe by a plunger driven by a constant speed motor. The concentration of fluid could be varied to effect a desired rate of administration per minute. The outlet of the syringe was fitted with a three-way stopcock which was then connected with a No. 22 blunt needle by means of intravenous rubber tubing. The air bubbles in the syringe and tubing were removed by filling and emptying them several times with the required dilution of standardized digitalis tincture. With this procedure the following dosage ranges of digitalis tincture were used: 0.0025, 0.0049, 0.0101, 0.0153, 0.0200, 0.0226, 0.0250, and 0.0400 cc./Kg./min. In each case the required dosage of the tincture was diluted to 15 cc. with normal saline solution at the time of the experiment.

Pigeons weighing 350–550 Gm. were given water *ad lib.* but were fasted for eighteen to twenty-four hours. The animals were anesthetized with an intramuscular injection of pentobarbital sodium (40 mg./Kg.).[2] The alar vein of the bird was then exposed and cannulated with a No. 22 gauge blunt needle. The injection was continued until the bird died in cardiac systole.

* Received August 22, 1952, from the College of Pharmacy, University of Washington, Seattle.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.
† This study has been supported in part by funds from the State of Washington Fund for Medical and Biological Research, University of Washington, and the American Foundation for Pharmaceutical Education.
‡ Present address: Department of Pharmacognosy, School of Pharmacy, Oregon State College, Corvallis.
§ Department of Pharmacology, College of Medicine, State University of New York, Syracuse.
[1] Drug Plant Laboratory, College of Pharmacy, University of Washington, Seattle.

[1] Supplied by the Department of Pharmacology, School of Medicine, University of Washington, Seattle.
[2] Although more convenient than ether anesthesia, it has been recognized by Kaplan and Visscher (8) that under this condition the lethal dose is increased by 41–50%.

TABLE I.—RATE OF CONTINUOUS ADMINISTRATION OF DIGITALIS TINCTURE AND THE LETHAL DOSE IN PIGEONS

Experiment	No. of Assays	Rate of Continuous Administration, Cc./Kg./Min.	Lethal Dose, Cc./Kg.	Experimental Time, Min.
A	6	0.0025	0.8544 ± 0.035	342 ± 5.84
B	5	0.0049	0.8647 ± 0.095	176 ± 19.50
C	6	0.0101	0.8937 ± 0.025	89 ± 4.40
D	5	0.0153	0.8907 ± 0.087	59 ± 5.75
E	5	0.0200	0.8842 ± 0.020	44 ± 1.41
F	4	0.0226	0.9365 ± 0.084	42 ± 2.65
G	5	0.0250	1.0314 ± 0.099	41 ± 3.50
H	5	0.0400	1.0564 ± 0.063	27 ± 1.41

RESULTS

The results of the administration of a standard digitalis tincture by a slow continuous injection method into the alar vein of 41 pigeons is shown in Table I. In Figs. 1 and 2, the rate of administration and experimental time are plotted against lethal dose, respectively. It can be seen that with the above pigeon method the lethal dose of digitalis tincture increased with the rate of administration. However, in experiments C, D, and E with a two-fold increase in rate from C to E, the closest correlation exists with an average lethal dose of 0.8828 cc./Kg. (0.88 cc.) and an experimental time of from forty-four to eighty-nine minutes. If this critical average is selected as the "smallest M. L. D." of Maresh and Farah (5), it will be observed that the rates of injection of A and B (0.0025 and 0.0049 cc./Kg./min.) were within a factor only 5% lower than the C-E average, but the experimental time is considerably greater in proportion. Furthermore, with the faster rate and larger dose (i. e., experiments F, G, and H), lethal doses ranged from 5 to 15% higher and at a relatively closer experimental time.

The fact that rather wide dosage rates and a wide range of experimental time (44–342 minutes) could be selected for experiments A-E without influencing the M. L. D. by more than ±5% indicates that with the continuous injection procedure and within these limits a rather consistent glycoside response takes place in pigeons. Furthermore, a rather broad lethal dose plateau apparently is reached during continuous administration of these rates. This plateau resembles that previously reported by Maresh and Farah (5) for purified digitalis glycosides.

It is difficult, therefore, to assess any significant differences between the lethal dose of experiments A through E in Table I, although the rates of administration and experimental time were varied. When compared with data reported by others (see Figs. 3 and 4) who employed cats (1–5), it appears that, following continuous injection of all of these slow rates of digitalis tincture into pigeons, the lethal dose curve becomes relatively constant at a broader range of experimental time than thus far found in the cat.

At slow rates of continuous injection such as the above, contrary to the suggestions of Haag and

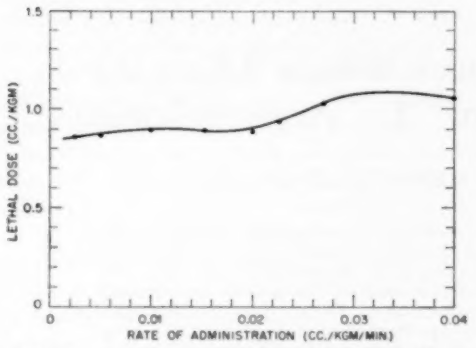


Fig. 1.—Intact anesthesized pigeon. Relation of rate of continuous administration of U. S. P. digitalis tincture to lethal dose.

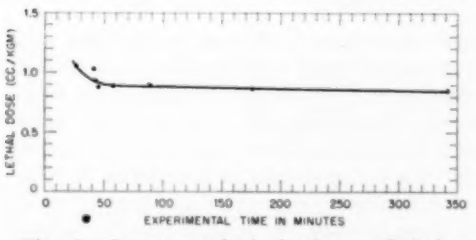


Fig. 2.—Intact anethesized pigeon. Relationship of experimental time to lethal dose of U. S. P. digitalis tincture following continuous administration.

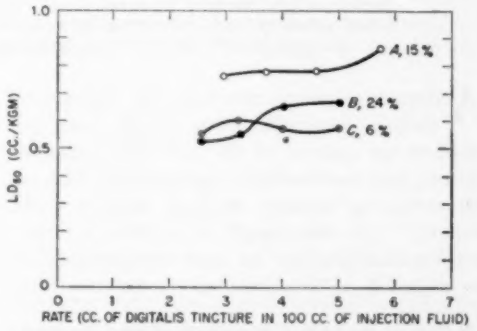


Fig. 3.—Chart modified from Bliss and Allmark (3) showing relationship of rate of administration to LD_{50} of digitalis tincture in the anesthesized cat. U. S. P. XII cat assay. *A*, *B* and *C* = digitalis tincture of 3 different commercial sources.

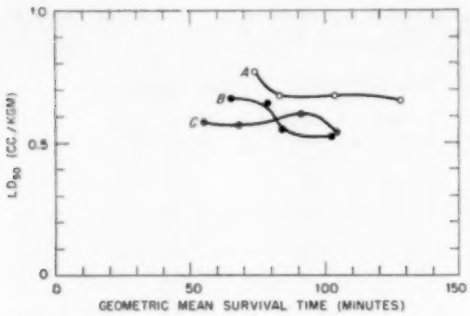


Fig. 4.—Chart modified from Bliss and Allmark (3) showing relationship of experimental time to LD_{50} of digitalis tincture in anesthesized cats. U. S. P. XII cat assay.

Woodley (6), it would appear that there is little or no effect on the M. L. D. range such as would be caused by the elimination of glycosides (i. e., from their fixation, excretion, or other similar factors) in the pigeon.

SUMMARY

The continuous administration of a wide range of rather slow injections of digitalis tincture has been studied in pigeons.

There was no significant change in the lethal dose produced by dose levels of from 0.0025 to 0.0226 cc./Kg./min. A wide range of experimental time (42–340 minutes) can be employed for conducting the assay in pigeons in contrast to that reported for cats.

REFERENCES

(1) Kingisepp, G., and Lindle, L., *Arch. exptl. Path. Pharmakol.*, **187**, 106(1937).
(2) Dawson, W. T., Buckley, H., and Gardner, H. L., *J. Pharmacol. Exptl. Therap.*, **66**, 8(1939).
(3) Bliss, C. I., and Allmark, M. G., *ibid.*, **81**, 378(1944).
(4) Matuda, K., Tazawa, T., Hasimoto, K., and Nukada, A., *Jap. J. Med. Sci. IV, Pharmacol.*, **11**, 6(1938).
(5) Maresh, G. J., and Farah, A. E., *J. Pharmacol. Exptl. Therap.*, **90**, 304(1947).
(6) Haag, H. B., and Woodley, J. D., *ibid.*, **51**, 360(1934).
(7) "The United States Pharmacopeia," Fourteenth Revision, Mack Publishing Co., Easton, Pa., 1950, p. 179.
(8) Kaplan, J. J., and Visscher, M., *J. Pharmacol. Exptl. Therap.*, **70**, 228(1940).

The Structure of Substances Which Affect the Autonomic Nervous System. I. Arylvinylamines*

By GEORGE P. HAGER and KENNETH H. STAHL†

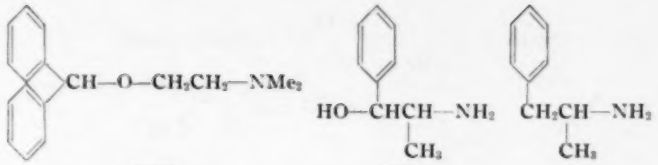
A working hypothesis concerning the relationship of structure and activity of substances which affect the activity of the autonomic nervous system has been drawn up. It is based on the orientation of functional groups and substituents in the molecules of various pharmacodynamically active substances and on the known or assumed biological transformation of certain of the substances. Two arylvinylamines, prepared in an attempt to probe the reality of the hypothetical considerations, have been shown in preliminary pharmacologic tests to produce a rather prolonged fall in blood pressure and, following dibenamine, to exert a profound stimulatory effect of considerable duration on both the rate and amplitude of respiration.

A NUMBER of pharmacodynamically active substances, especially those which have an effect on the activity of the autonomic nervous system, may be classified on a structural basis as derivatives of α-amino alcohols such as colamine (I). In what might be a more comprehensive classification, the same compounds may be regarded as 2-substituted-ethylamines (II).

$$\underset{\text{(I)}}{HO{-}CH_2CH_2{-}NH_2} \qquad \underset{\text{(II)}}{X{-}CH_2CH_2{-}NH_2}$$

Diphenhydramine (III) may be pictured as a derivative of either type, a benzhydryl ether of colamine or a benzhydryloxy-derivative of ethylamine. Propadrine (IV), on the other hand, may be most conveniently classified as a derivative of a propanolamine (a homolog of I), while amphetamine (V) is best classified as a derivative of isopropylamine (a homolog of II). In any case, the nature of the substituent on the carbon atom adjacent to that which bears the amino group has a profound effect on the pharmaco-

* Received August 22, 1952, from the School of Pharmacy, University of Maryland, Baltimore.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.
† Instructor, Department of Pharmaceutical Chemistry, School of Pharmacy, University of Maryland, 1947–1951. Present address: Detroit Institute of Technology, College of Pharmacy, Detroit, Mich.



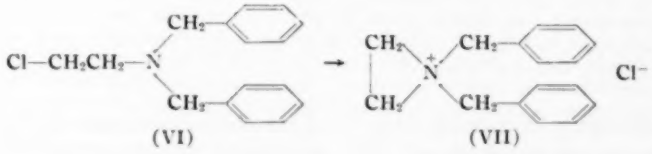
(III) (IV) (V)

dynamic properties of the compound, i. e., the group X in compounds of type II is an important determinant of the pharmacodynamic properties. Such properties are also influenced, especially in a quantitative manner, by the nature and the number of substituents on the amino group.

The necessity for an adjacent relationship of the amino group and the group X is apparent in a number of typical agents which affect the activity of the autonomic nervous system. Such is the case with sympathomimetic amines (1, 2), sympatho-adrenolytic 2-chloroalkylamines (3), parasympathomimetic choline esters, and related parasympatholytic agents (4). The histaminolytic activity of ethers of aminoalcohols is also maximum when aralkoxy and amino groups are on adjacent carbon atoms as in diphenhydramine (5, 6). In the other important series of histaminolytic agents, the ethylenediamine derivatives and related compounds, the substituents are again on adjacent carbon atoms. An ethylene group linking a tertiary amino group to a quaternary carbon atom is a structural feature common to the analgesics, morphine, meperidine, and methadon (7), and such a grouping is consistent with a pattern of adjacent orientation of functional groups or substituents.

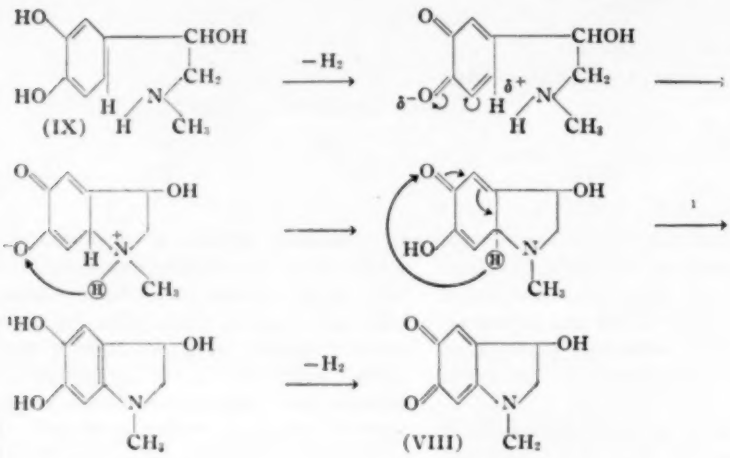
The necessity for an adjacent orientation of a group X (formula II) and an amino group in compounds with maximum pharmacodynamic activities of various types appears to have been well established empirically. Theoretical considerations in an effort to explain the necessity for this structural feature have been advanced for the adrenergic-blocking agents (3) and for the muscarinic and cholinergic-blocking agents (4). The dissimilarity of the two hypotheses is striking and Nickerson (3) was prompted to suggest that the chemical basis established for the adrenergic-blocking activity of 2-chloroalkylamines should "serve to focus attention upon the importance of the *chemical* properties of pharmacologically active agents, which in the past have been almost completely neglected in favor of *physical* (steric) properties." The possibility that a chemical basis and a physical basis for activity might be coexistent cannot be denied. On the other hand, a common basis, be it chemical or physical, in keeping with the common necessity for an adjacent orientation of groups in the molecular structure, might resolve a number of incongruities now apparent in the structure-activity relationships of the pharmacodynamically active substances here under consideration. As an example of such incongruities, the somewhat anomalous effect of orientation of phenolic hydroxyls on the pressor activity of phenylalkanolamines might be mentioned.

In a working hypothesis which serves as a guide in the present investigation, the assumption has been made that the adjacent orientation of substituents on an alkylene group makes possible an interaction between the amino group and the group X (formula II) or the carbon atom bearing group X that may vary from a field effect through chelation to covalency. It is further assumed that such an interaction is prerequisite for a pharmacodynamic effect. An interaction of this type in which a covalent bond is formed has been described in explanation of the activity of 2-chloroalkylamines (3). In the case of dibenamine (VI), the N,N-dibenzylethylenimmonium ion (VII) is formed as a result of this interaction (Eq. 1) and through its effect as an electrophilic alkylating agent, it prevents certain responses to epinephrine and to sympathetic stimulation.



(VI) (VII) (1)

A somewhat similar interaction appears to be involved in the formation of adrenochrome (VIII)



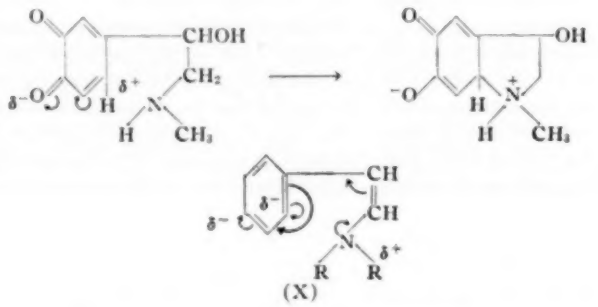
[1] A dienone-phenol tautomerism.

from epinephrine (IX), a transformation that may follow a course suggested in Eq. 2.

A possible extension of the general idea of intramolecular interaction of the amino group with the group X or with the adjacent carbon atom by a reaction which involves the group X in explanation of the pharmacodynamic activity of other substances having the same structural feature (formula II) seems obvious.

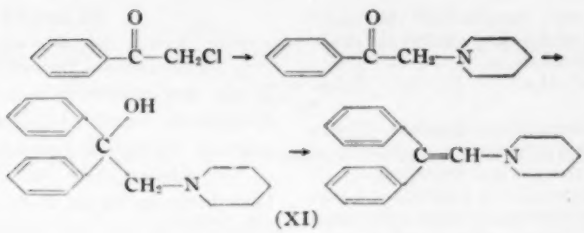
One approach in probing this hypothesis was suggested by the following observation. The electrostatic interaction of the amino group and the group X in all of the series of pharmacodynamically active substances considered above can in no instance be propagated through the intervening alkylene group because the conjugation is broken by a sequence of three single bonds. The alkylene group acts only in a passive way by providing a proper orientation for interaction between the two groups. If the above assumptions concerning a chemical basis for pharmacodynamic activity are based on a reality, then it might be expected that an interaction of the amino group and the group X which is propagated through an unsaturated alkylene group would result in pharmacodynamic activity in the compound. In the following formulas, an analogy between the intramolecular interaction of groups in an intermediate in the above reaction suggested for the formation of adrenochrome and that of the groups in the "unsaturated" analog (X) is suggested (Eq. 3).

In both cases the nitrogen atom of the amino group becomes electrophilic as a result of the interaction. In this regard, the fact that certain of the pharmacodynamic effects of epinephrine are blocked by the electrophilic N,N-dibenzylethylenimmonium ion may have some significance.



Two substituted vinylamines related to (X) have been prepared as indicated by Eq. 4.

By this means 1,1-diphenyl-2-(1-piperidino)-ethylene (XI) and 1,1-diphenyl-2-(4-morpholino)-ethylene have been prepared and iso-



(4)

(XI)

lated as their hydrochlorides. In attempts to prepare 1,1-diphenyl-2-diethylamino-ethylene and 1,1-diphenyl-2-di-*n*-butylamino-ethylene, deamination rather than dehydration occurred in the final step. Current investigations have as their objective the preparation of these two compounds and other members of the series as well as a series of 1-phenyl-2-substituted-amino-ethylenes and a series of 1-phenyl-1-alkyl-2-substituted-amino-ethylenes.

Preliminary pharmacologic tests showed that the substituted vinylamines (XI), administered as their hydrochlorides, have an effect on blood pressure as well as on respiration. Insufficient information is available at this time. for any conclusions as far as structure-activity relationships are concerned.

EXPERIMENTAL

All melting points are uncorrected and were determined with a Fisher-Johns apparatus.

ω-(1-Piperidino)-acetophenone.—This compound was prepared from phenacyl chloride by reaction with piperidine in benzene in the presence of anhydrous potassium carbonate in 59% yield as directed by Henley and Turner (8).

ω-(4-Morpholino)-acetophenone Hydrochloride.—This compound was prepared from phenacyl chloride by reaction with freshly distilled morpholine in ethanol in 61.5% yield as directed by Rubin and Day (9), m. p. 215–216° (dec.), reported m. p., 223–224° (9), 213–214° (10).

1,1-Diphenyl-2-(1-piperidino)-1-ethanol.—A solution of phenylmagnesium bromide in anhydrous ether was prepared from 122.5 Gm. (0.78 mole) of bromobenzene and 38 Gm. (1.56 gram-atoms) of magnesium in the usual manner (11). The solution of the Grignard reagent was decanted from the excess magnesium into a three-neck flask fitted with a mercury-seal stirrer, reflux condenser, and dropping funnel. A solution of 80 Gm. (0.39 mole) of ω-(1-piperidino)-acetophenone in 250 cc. of anhydrous ether was added dropwise with stirring at such a rate that ether refluxed without the application of heat. When the addition was complete, the mixture was refluxed gently for one hour, cooled, and treated with a mixture of cracked ice, 300 Gm. of ammonium chloride, and 1,000 cc. of water. The ether layer was separated and extracted with dilute hydrochloric acid. The acid extract was washed with ether, made alkaline with ammonia solution, and extracted with ether. The ether extract was washed with water, dried with anhydrous sodium sulfate, and filtered. 1,1-Diphenyl-2-(1-piperidino)-1-ethanol hydrochloride precipitated as a reddish semi-solid when dry hydrogen chloride was passed into the ether solution, and was recrystallized from a mixture of absolute alcohol and anhydrous ether as white crystals, m. p. 223–224° (dec.), reported m. p. 214–218° (8), in a yield of 55 Gm. (51%).

The free base was obtained from the hydrochloride by treatment with sodium hydroxide, and recrystallized from dilute alcohol as white crystals, m. p. 67–68°. This was used in subsequent experiments without further purification.

1,1-Diphenyl-2-(4-morpholino)-1-ethanol.—ω-(4-Morpholino)-acetophenone was obtained from the corresponding hydrochloride by treatment with sodium hydroxide solution, extraction with ether, drying the ether solution with anhydrous sodium sulfate, and removal of the ether by distillation under reduced pressure. It was treated with an ether solution of phenylmagnesium bromide as described in the preparation of 1,1-diphenyl-2-(1-piperidino)-1-ethanol. After hydrolysis of the reaction mixture, the ether solution was extracted with dilute hydrochloric acid. The acid extract was made alkaline with sodium hydroxide solution and cooled. The 1,1-diphenyl-2-(4-morpholino)-1-ethanol which precipitated was recrystallized from 70% ethanol as white crystals, m. p. 77–78°, in a yield of 17.5 Gm. (33% of theoretical). This was used without further purification in the subsequent dehydration reaction.

1,1-Diphenyl-2-(1-piperidino)-ethylene Hydrochloride.—In an attempt to dehydrate 1,1-diphenyl-2-(1-piperidino)-1-ethanol by refluxing a solution of 0.015 mole of the compound in a mixture of 15 cc. of hydrochloric acid and 50 cc. of glacial acetic acid for three hours, piperidine hydrochloride was the only product isolated, indicating that deamination rather than dehydration had taken place. This method was described for the dehydration of similar amino alcohols in the preparation of various substituted allylamines (12).

A method for dehydration of diphenylcarbinols to such compounds as 1,1-diphenyl-4-diethylamino-1-butene (13) was applied successfully in the dehydration of 1,1-diphenyl-2-(1-piperidino)-1-ethanol. A solution of 0.006 mole of the compound in 20 cc. of acetic anhydride was refluxed for fifteen minutes. If the mixture were refluxed for longer periods of time, deamination and formation of piperidine hydrochloride resulted. The excess acetic anhydride and any acetic acid present were removed by distillation under reduced pressure. The residue was dissolved in ether, and anhydrous potassium carbonate was added; after standing for thirty minutes, the solution was filtered. Dry hydrogen chloride was passed into the ether solution and the 1,1-diphenyl-2-(1-piperidino)-ethylene hydrochloride

which precipitated was recrystallized in 52.5% yield from a mixture of absolute alcohol and anhydrous ether as white crystals, m. p. 186–187° (dec.).

Anal.—Calcd. for $C_{19}H_{22}ClN$: N, 4.67. Found: N, 4.48, 4.55.

1,1-Diphenyl-2-(4-morpholino)-ethylene Hydrochloride.—1,1-Diphenyl-2-(4-morpholino)-1-ethanol was dehydrated by the method described for the dehydration of 1,1-diphenyl-2-(1-piperidino)-1-ethanol with the exception that the mixture was refluxed for one and one-half hours; white crystals, m. p. 154–155°, 41.7% yield, were obtained.

Anal.—Calcd. for $C_{18}H_{20}ClNO$: N, 4.64. Found: N, 4.64, 4.63.

PHARMACOLOGY[1]

In a preliminary test, the effects of the hydrochlorides of 1,1-diphenyl-2-(1-piperidino)-ethylene (XI) and 1,1-diphenyl-2-(4-morpholino)-ethylene (XII) on the blood pressure (carotid) and respiration (tracheal) were observed on two dogs (Dial anesthesia). In doses up to 50 mg. (total), both compounds produced a pronounced fall in blood pressure of rather long duration followed by a return to normal levels. The effect on blood pressure was not altered in any way by atropine, dibenamine, ergotoxine, or diphenhydramine. The preliminary tests seem to indicate that the compounds produce a vasomotor response which differs from that elicited by acetylcholine, histamine, and epinephrine. Further pharmacologic investigation is being carried out to identify more precisely the site of action of these compounds in producing a vasomotor response.

About one hour after the administration of dibenamine, a 50-mg. dose of the compounds produced, concurrently with the effects on blood pressure, what appeared to be a maximum stimulation of the respiration in regard to both depth and frequency, an effect which lasted for an unusually long period (at least thirty minutes). It is possible that pharmacologic studies now in progress will reveal that the effect of the compounds on respiration will be of considerably greater significance than their effects on blood pressure.

[1] Preliminary pharmacologic studies were carried out by Mr. Gordon H. Bryan, Department of Pharmacology, School of Pharmacy, University of Maryland, Baltimore.

SUMMARY

1. On the basis of the orientation of functional groups and substituents in various pharmacodynamically active substances and the known or assumed biological transformations of certain of the substances, a working hypothesis has been drawn up for the study of structure-activity relationships and the possible development of new drugs affecting the activity of the autonomic nervous system.

2. The preparation of arylvinylamines has been one of the initial steps in probing the reality of the hypothetic considerations, and the preparation of 1,1-diphenyl-2-(1-piperidino)-ethylene and 1,1-diphenyl-2-(4-morpholino)-ethylene has been described.

3. The hydrochlorides of both of the substituted vinylamines, in preliminary pharmacologic tests, were found to produce a rather prolonged fall in blood pressure and to exert a profound effect on both the rate and the amplitude of respiration after pretreatment with dibenamine. The latter effect was especially striking because of its long duration.

REFERENCES

(1) Barger, G., and Dale, H. H., *J. Physiol.*, **41**, 19(1910).
(2) Graham, B. E., and Cartland, G. F., *J. Pharmacol. Exptl. Therap.*, **81**, 360(1944).
(3) Nickerson, M., and Gump, W. S., *ibid.*, **97**, 25 (1949).
(4) Pfeiffer, C. C., *Science*, **107**, 94(1948).
(5) Haley, T. J., THIS JOURNAL, **37**, 383(1948).
(6) Loew, E. R., Kaiser, M. E., and Moore, V., *J. Pharmacol. Exptl. Therap.*, **83**, 120(1945).
(7) Eddy, N. B., THIS JOURNAL, **39**, 245(1950).
(8) Henley, R. V., and Turner, E. E., *J. Chem. Soc.*, **1931**, p. 1182.
(9) Rubin, N., and Day, A. R., *J. Org. Chem.*, **5**, 57 (1940).
(10) Chemische Werke vorm H. & E. Albert, Ger. pat. 667,356, Nov. 9, 1938.
(11) Fieser, L. F., "Experiments in Organic Chemistry," 2nd ed., D. C. Heath and Co., New York, 1941, pp. 68–69.
(12) Adamson, D. W., *J. Chem. Soc.*, Suppl. Issue, No. 1, **1949**, S144.
(13) Marxer, A., *Helv. Chim. Acta*, **24**, 209E(1941).

The Synthesis of Certain Sulfur-35 Labeled Sulfa Drugs*,†

By P. J. BYRNE, Jr., A. A. ALBERTS, and J. E. CHRISTIAN

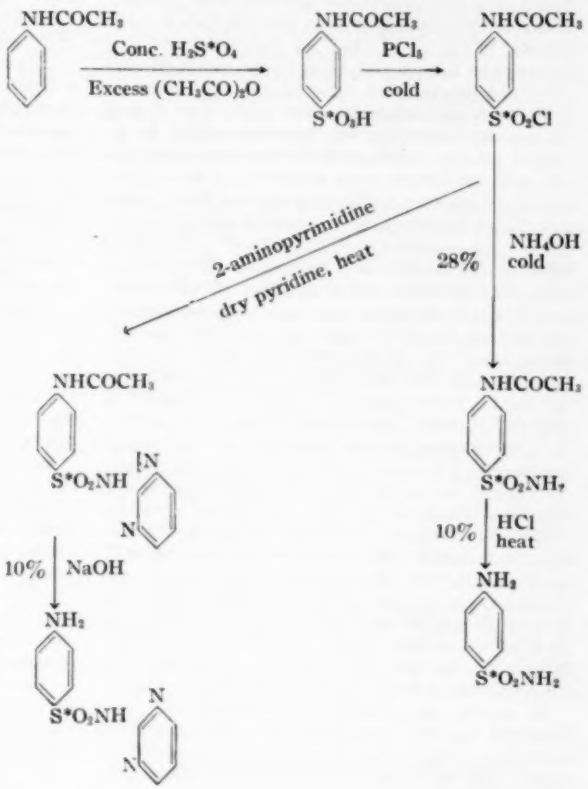
The synthesis of isotopic labeled compounds involves somewhat different techniques and methods than usual chemical syntheses. This paper describes the procedures used to synthesize sulfur-35 labeled N^4-acetylsulfanilamide, sulfanilamide, 2(N^4-acetyl sulfanilamido)pyrimidine, and 2-sulfanilamidopyrimidine.

BECAUSE of the continued use of the sulfa drugs and, in particular, mixed sulfa drug therapy, it is important toattempt togain further insight into the mode of action, metabolism, and excretion processes of the drugs. In addition, the problem of mixed sulfa analysis in pharmaceutical preparations and in biological fluids where small quantities are present is of considerable importance.

The development of radioisotope tracer techniques in recent years and their extensive application to biological and analytical problems have suggested the use of sulfur-35 labeled sulfa drugs for the study of many problems involving these compounds. Accordingly, methods are reported in this paper for the synthesis of four of the sulfa drugs labeled with sulfur-35.

Careful consideration was given to two sources of information available on the synthesis of sulfur-35 labeled sulfanilamide. Some of this information served as a basis for the syntheses of the compounds described in this report. Fingl, Christian, and Edwards (1) reported briefly on their method of synthesis, and Klotz and Melchior (2) reported a synthesis by essentially the same method. In this work, a portion of the procedures used is similar to that previously described by these workers but with some modifications and extensions.

The method of synthesis is outlined by the following equations:



The labeled sulfuric acid used in this work was obtained from the atomic energy pile at Oak Ridge, Tenn. Other materials are standard chemical reagents.

EXPERIMENTAL

N-Acetylsulfanilyl Chloride.—Sulfur-35 labeled sulfuric acid supplied in a dilute solution of hydrochloric acid and with an activity of approximately 2 mc. was transferred to a 30-ml. beaker and evaporated to dryness by an infrared lamp. Two

* Received August 22, 1952, from the Department of Chemistry and the School of Pharmacy, Purdue University, Lafayette, Ind.

Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.

† The isotopes used in this research were received on allocation from the United States Atomic Energy Commission.

milliliters of concentrated sulfuric acid was added as carrier, and the acid was cooled while 4 ml. of acetic anhydride was added slowly. Following this, 2 Gm. of acetanilide was added in small portions with good mixing and the resultant mixture was heated to 93–100° on a steam bath for thirty minutes; N-acetylsulfanilic acid separated. The reaction mixture was cooled in an ice bath, diluted with 10 ml. of chilled acetone, filtered on a Büchner funnel with suction, washed with a small quantity of chilled acetone, transferred to a porous clay plate, and dried under an infrared lamp. Yields of 2.99 Gm. (94.05%, based upon the acetanilide) and 2.84 Gm. (89.31%, based upon the acetanilide) of the crude N-acetylsulfanilic acid were obtained on successive runs.

While chilling, 2.99 Gm. of N-acetylsulfanilic acid was rubbed with 5.98 Gm. of phosphorus pentachloride. After the initial reaction had subsided, the mixture was gently heated until a homogeneous liquid mass indicated the completion of the reaction. The solution was cooled in an ice bath and approximately 10 ml. of crushed ice was then added to decompose the excess phosphorus pentachloride. The precipitate was N-acetylsulfanilyl chloride.

N^4-Acetylsulfanilamide.—Ten milliliters of 29% ammonium hydroxide was carefully added to the chilled solution containing N-acetylsulfanilyl chloride, and the mixture was warmed on a steam bath for thirty minutes. The solution was then cooled and dilute sulfuric acid was slowly added until a *p*H of approximately 3 was obtained. The chilled mixture was filtered on a Büchner funnel with suction, washed with a small quantity of cold water, transferred to a porous clay plate, and dried under an infrared lamp. A yield of 1.64 Gm. (54.8%, based upon the N-acetylsulfanilic acid) of crude N^4-acetylsulfanilamide, melting at 214–218°, was obtained. Recrystallization from water gave 1.29 Gm. (43.1%) of N^4-acetylsulfanilamide; m. p. 219°.

To determine the radioactivity of N^4-acetylsulfanilamide, a sample weighing 51 mg. was evenly distributed on the bottom of a glass counting cup having a diameter of 12.7 mm. (area of about 1.27 cm^2). This gave a sample thickness of 40.1+ mg./$cm.^2$ Since the saturation thickness of S-35 is approximately 16 mg./$cm.^2$, the sample was present in saturation thickness. Under these conditions the sample was observed to have an activity of 4,567 corrected counts per minute at 4.5 cm. from a Tracer Lab. TGC 1-1883 end window tube having a mica window thickness of 2.7 mg./$cm.^2$

A second portion of N^4-acetylsulfanilic acid (2.48 Gm.) carried through this same process yielded 2.13 Gm. (75.0% based upon the N-acetylsulfanilic acid) of crude N^4-acetylsulfanilamide melting at 213–216°.

Sulfanilamide.—Crude N^4-acetylsulfanilamide (2.13 Gm.) was hydrolyzed by warming on a steam bath in 5 ml. of a 10% hydrochloric acid solution for one hour. The mixture was cooled and then neutralized in two steps. The first step consisted of bringing the solution to a *p*H of approximately 5 with 5 *N* sodium hydroxide solution. In the final step, a 10% solution of sodium bicarbonate was used to complete the neutralization of the solution. The chilled solution was filtered on a Büchner funnel with suction, washed with a small quantity of cold water, transferred to a porous clay plate, and dried under an infrared lamp. A yield of 1.04 Gm. (36.6% based on the N-acetylsulfanilic acid) of sulfanilamide melting at 164° was obtained after recrystallization from water.

A sample weighing 74 mg. was evenly distributed on the bottom of a glass counting cup and counted as previously described. An activity of 3,727 corrected counts per minute was observed.

2-(N^4-Acetylsulfanilamido)pyrimidine.—A sample of 1.05 Gm. of 2-aminopyrimidine was suspended in 5 ml. of dry pyridine. To the chilled mixture, 2.98 Gm. of dry crude labeled N-acetylsulfanilyl chloride (prepared as previously described) was added gradually, with stirring, at such a rate that the temperature did not exceed 55°. The reaction mixture was heated on a steam bath for one hour. The pyridine was removed under diminished pressure in the temperature range of 27–42°, water being added from time to time to maintain a minimum volume of about 25 ml. A yield of 1.50 Gm. (46.5% based upon the 2-aminopyrimidine) of the crude 2-(N^4-acetylsulfanilamido)pyrimidine separated as a yellow solid which melted at 217°.

A second portion of 2.25 Gm. of dry crude labeled N-acetylsulfanilyl chloride and 0.82 Gm. of 2-aminopyrimidine was condensed in 5 ml. of pyridine and yielded 0.95 Gm. (37.6% based upon the 2-aminopyrimidine) of the crude 2-(N^4-acetylsulfanilamido)-pyrimidine melting at 218°. This was recrystallized from 60% acetic acid to give a product melting at 257–258°.

A sample weighing 27 mg. was prepared for counting as described and was observed to have an activity of 3,692 corrected counts per minute.

2-Sulfanilamidopyrimidine.—2-(N^4-Acetylsulfanilamido)pyrimidine (1.50 Gm.) was dissolved in 4.5 ml. of 10% sodium hydroxide and refluxed for one and one-half hours. The reaction mixture was then cooled, diluted with 20 ml. of cold water, filtered, and neutralized with dilute hydrochloric acid. The white precipitate that formed was collected by filtration, washed with cold water, and dried under an infrared lamp. A yield was obtained of 1.12 Gm. (34.7% based upon the 2-aminopyrimidine) of crude 2-sulfanilamidopyrimidine melting at 218°. This product, after recrystallization first from 60% acetic acid and finally from a 50% ethanol-water mixture, had a melting point of 255°.

A sample weighing 62 mg. was observed to have an activity of 6,231 corrected counts per minute as determined by the method described.

The radioactive purity of the labeled compounds synthesized was proved by ion-exchange chromatography following the procedures described by Hutchins and Christian (3).

SUMMARY

Starting with acetanilide and sulfur-35 labeled sulfuric acid, methods for the synthesis of N^4-acetylsulfanilamide, sulfanilamide, 2-(N^4-acetylsulfanilamido)pyrimidine, and 2-sulfanilamidopyrimidine labeled with sulfur-35 have been described in detail. The methods employed here may be applied to the synthesis of other labeled sulfa drugs for utilization in fundamental research problems.

REFERENCES

(1) Fingl, E. G., Christian, J. E., and Edwards, L. D., THIS JOURNAL, **39**, 693(1950).

(2) Klotz, I. M., and Melchior, J. B., *Arch. Biochem.*, **21**, 35(1949).

(3) Hutchins, H. H., and Christian, J. E., THIS JOURNAL, in press.

A Preliminary Study of Silicone Oils as Dermatological Vehicles*,†

By JOY BICKMORE PLEIN and ELMER M. PLEIN‡

An investigation was made of the pharmaceutical properties of silicone oils in dermatological preparations. The oils were mixed with a series of acids, alcohols, waxes, and commercial ointment bases to determine their compatibilities. Silicone oil emulsions and suspensions were prepared, and the use of silicones in liniments was studied. Six silicone ointment bases, 3 U. S. P. ointment bases, and 17 commonly prescribed medicinal agents were used in preparing a series of ointments for aging and compatibility tests. The diffusion rates of some medicinal agents from these silicone bases and official ointment bases were determined *in vitro*.

WITHIN the past few years, the silicones have found a place in nearly every industry and the list of uses for these versatile synthetics is increasing daily. Friedel, Crafts, and Landenburg (1) prepared organic compounds of silicon a hundred years ago, and Kipping and his coworkers (1) published fifty papers dealing with organosilicon compounds, but significant practical use of silicones was not realized until the later years of the Second World War (2). In pharmacy and medicine, silicones have been used to provide water-repellent films for preventing the adherence of liquids to glassware, parenteral equipment, and containers for pharmaceuticals (3–6). A patent has been issued for the use of a silicone-resin coating for capsules, pills, and tablets to provide protection from moisture and oxidation (7). Their use as pharmaceutical vehicles has not been reported.

Silicone oils are mixtures of polymers of dimethylsiloxane (1). They are sparkling clear liquids, odorless and tasteless, insoluble in water, and highly water repellent (8). Their viscosity is dependent on the average polymer chain length (1), and the fluids are produced in a viscosity range from 0.65 to 1,000,000 centistokes (8). In general, the solubility of silicone oils is that of nonpolar liquids (8). Useful common solvents for the oils are benzene, chloroform, ethyl oxide, amyl acetate, and carbon tetrachloride. Properties which are especially interesting pharmaceutically are the extremely small change of viscosity with thermal change and the high oxidation resistance and chemical stability possessed by these compounds (8).

The silicone oils, except for hexamethylsiloxane (DC 200, 0.65 centistokes) are nontoxic. No appreciable irritation resulted from intraperitoneal and intradermal injection, and the oral toxicity is very low (9, 10). None of the oils caused any skin irritation upon local application, but if they were rubbed into the eyes, a transitory conjunctival irritation developed which disappeared within twenty-four hours and left no corneal damage (9). This would, of course, eliminate the silicones for use in ophthalmic preparations.

EXPERIMENTAL AND DISCUSSION

The silicone oils used in this investigation were Dow Corning dimethylsiloxane polymers (DC 200 Fluids).

Compatability of Silicone Oils with Other Dermatological Vehicles.—In order to determine whether or not the silicone oils could be mixed with other substances used in formulating vehicles for dermatological preparations, silicone oils of 50-, 1,000-, and 200,000-centistoke viscosities were mixed with a series of 42 acids, alcohols, waxes, and commercial ointment bases. Three oils widely varying in viscosity were selected because there is some difference in the solubility properties of oils of different

* Received May 15, 1952, from the College of Pharmacy, University of Washington, Seattle.

Presented to the Section on Practical Pharmacy, A. PH. A., Buffalo meeting, August, 1951.

† Abstracted from a thesis submitted to the Graduate School of the University of Washington by Joy Bickmore in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.

‡ Professor of Pharmacy, College of Pharmacy, University of Washington.

viscosities (8), and it was thought their compatibilities with other bases might also vary. Results indicated, however, that differences in the stability of the various binary mixtures were probably due to differences in viscosity properties only. The high-viscosity oil produced more stable mixtures than did the low-viscosity oils. Cetyl alcohol, theobroma oil, diglycol stearate S, glyceryl monostearate, hydroxystearin sulfate, lauric acid, wool fat, polyethylene glycol 400 monostearate, stearic acid, stearyl alcohol, and Unibase produced good binary mixtures with the dimethylsiloxane polymers. As would be expected, the water-soluble substances—carbowaxes, soft soap and glycerin—would not form stable mixtures with the silicone. It was difficult to mix paraffin and waxes—Alrowax 75,[1] Alrowax 140,[1] carnauba wax, ceresin wax, Forbex Wax 44,[2] spermaceti, and white wax—with silicone oils without separation of paraffin or the wax into definite granules; even when the combination was triturated in a mortar at a temperature above the melting point of the wax, the wax often crystallized when the mixture was cooled. Silicone oils were practically immiscible with vegetable oils, liquid petrolatum, some of the acids, and some of the alcohols, including cholesterol. The fact that the dimethylsiloxane polymers would not produce stable binary mixtures with these substances did not mean that they could not be used together if another substance were added to stabilize the mixture.

Emulsifiers for Silicone Oils.—A series of emulsions of silicone oils (viscosity grades 10, 100, and 30,000 centistokes) and water was prepared by using various emulsifying agents.

Silicone emulsions can be prepared with the gums commonly used in pharmaceutical practice. Emulsions produced by acacia, using the Continental 4:2:1 method, were fair. With tragacanth, an emulsion of 10-centistokes oil could not be produced, whereas a smooth, white emulsion of 100-centistokes oil was formed with as little as 2% of the agent. Methyl cellulose emulsified or suspended the silicone poorly even when it was used in combination with another emulsifier.

The best emulsions in the series were those produced by a combination of G-2125[3] (28%) and G-2126[4] (72%). As suggested by the Atlas Powder Company (11), 4% of this agent produced white, O/W emulsions which did not separate quickly, and which mixed immediately upon agitation. The combination of G-2125 and G-2126 will be referred to as G Emulsifier. The emulsifier was mixed with the silicone oil, and water was slowly added while the mixture was agitated until the primary emulsion was formed.

The Atlas Powder Company suggested also an emulsifier composed of equal parts of BRIJ 30[5] and BRIJ 35[6] (11). This combination was not nearly as effective as was G Emulsifier. A mixture of equal parts of Tween 20[7] and Span 80[8] proved to be a good emulsifier for silicone oils.

Alrosol O,[9] Alrosol S,[9] Antarox B-290,[10] Antarox D-100,[10] Antarox A-200,[10] Antarox B-100,[10] Antaron L-135,[10] Antaron L-245,[10] and an emulsifier composed of dioctyl sodium sulfosuccinate, oleic acid, and triethanolamine gave only fair or poor emulsions of silicone and water. Antarox A-400[10] produced a poor emulsion of 10-centistokes oil, but its emulsions of 100- and 30,000-centistoke oils were good.

Formulation of Silicone Ointment Bases.—Several silicone-oil modifications of hydrophilic ointment U. S. P.; hydrophilic petrolatum U. S. P.; rose water ointment U. S. P. (12), Gibson base (13, 14), and glyceryl monostearate base (13, 15) were made and tested. These tests included a determination of the stability of the base, its general appearance, and ease of application. Modifications of official and recognized formulas were used because these well-known preparations are satisfactory, and because the substitution of silicone oils for the petrolatum and expressed almond oil which are used in these formulas would serve as a good comparison. In some cases a combination of a low-viscosity oil and a high-viscosity oil produced a better base than could be formed from a silicone of intermediate viscosity alone.

The following six ointment bases were selected from this series for further tests.

1. Silicone Hydrophilic Ointment

Stearyl Alcohol	20.0 Gm.
DC 200, 50 cts.	12.5 Gm.
DC 200, 1,000 cts.	12.5 Gm.
G Emulsifier	10.0 Gm.
Sodium lauryl sulfate	1.0 Gm.
Dist. water	44.0 Gm.
Methylparaben	0.25 Gm.
Propylparaben	0.15 Gm.

Melt the stearyl alcohol on a water bath, add the DC 200 Fluids, and warm the mixture to 75°. Add the other ingredients to the distilled water, and after warming the aqueous mixture to 75°, add it slowly and with agitation to the oily phase. Stir until the ointment congeals.

G Emulsifier is not necessary for emulsion formation; however, it serves as a better humectant than either glycerin or propylene glycol in this particular combination. This ointment is soft, white, and very smooth. It dries out less than does official hydrophilic ointment, but it becomes glossy with age.

2. Silicone Cold Cream

Spermaceti	10.0 Gm.
White wax	10.0 Gm.
Span 80	3.8 Gm.
DC 200, 100 cts.	25.0 Gm.
DC 200, 200,000 cts.	25.0 Gm.
Sodium borate	0.5 Gm.
Dist. water	14.5 Gm.
Glycerin	5.0 Gm.
Tween 20	6.2 Gm.

Melt the spermaceti and white wax on a water bath, add the Span 80 and silicone oils, and warm the mixture to 75°. Heat the aqueous mixture of the other

[1] Alrose Chemical Co., Providence, R. I.
[2] Forbex Corporation, 95 Broad St., New York 4, N. Y.
[3] G-2125 (Polyoxyethylene-4-laurate, Atlas Powder Co.).
[4] G-2126 (Polyoxyethylene-6-laurate, Atlas Powder Co.).
[5] BRIJ 30 (Polyoxyethylene lauryl alcohol, Atlas Powder Co.).
[6] BRIJ 35 (Polyoxyethylene lauryl alcohol, Atlas Powder Co.).
[7] Tween 20 (Polyoxyethylene sorbitan monolaurate, Atlas Powder Co.).
[8] Span 80 (Sorbitan monooleate, Atlas Powder Co.).
[9] Fatty acid emulsifiers, Alrose Chemical Co., Providence R. I.
[10] Products of Antara Products, 444 Madison Ave., New York 11, N. Y.

ingredients and pour it with stirring into the oily phase. Stir the ointment until it congeals.

This ointment is difficult to prepare because of the incompatibility of the silicone oils with waxes, especially beeswax. It is stiff, white, and has a tendency for very slight oil separation.

3. Silicone Anhydrous Base

Cholesterol	3.0 Gm.
Stearyl alcohol	3.0 Gm.
Carnauba wax	10.0 Gm.
Arlacel C	10.0 Gm.
Propylene glycol	10.0 Gm.
DC 200, 100 cts.	32.0 Gm.
DC 200, 200,000 cts.	32.0 Gm.

Dissolve the cholesterol in the Arlacel C at a temperature slightly over 100°. Melt the carnauba wax in a Wedgewood mortar heated by a water bath; add the other ingredients and maintain the temperature at 86° while stirring the mixture. Then cool the ointment slowly and rub it with a pestle during the cooling process.

Silicone anhydrous base is a stiff, light amber ointment.

4. Silicone Absorption Base

Arlacel C	6.0 Gm.
Carnauba wax	20.0 Gm.
DC 200, 200,000 cts.	24.0 Gm.
DC 200, 1,000 cts.	40.0 Gm.
Wool fat	10.0 Gm.

The preparation of this ointment is very similar to that of silicone anhydrous base which it resembles in appearance, although more stable.

5. Silicone Gibson Base

Sodium lauryl sulfate	1.0 Gm.
Cetyl alcohol	15.0 Gm.
DC 200, 1,000 cts.	40.0 Gm.
Dist. water	43.0 Gm.
Methylparaben	0.25 Gm.
Propylparaben	0.15 Gm.

Warm the aqueous mixture of the sodium lauryl sulfate and the parabens to 75° and slowly add it to the warmed (75°) cetyl alcohol-DC 200 mixture. Stir the ointment until it congeals.

Silicone Gibson base is soft, smooth, and easily applied to the skin. It does not decompose, but it slowly loses water on standing, especially over long periods of time.

6. Silicone Glyceryl Monostearate Base

Glyceryl monostearate	11.5 Gm.
DC 200, 50 cts.	19.2 Gm.
DC 200, 200,000 cts.	19.2 Gm.
Dist. water	46.0 Gm.
G Emulsifier	4.0 Gm.
Methylparaben	0.25 Gm.
Propylparaben	0.15 Gm.

Melt the glyceryl monostearate, add the silicones, water, G Emulsifier, and parabens, and heat the mixture to 75°. Stir the mixture until the ointment emulsifies and then congeals. G Emulsifier is added as a stabilizer.

This ointment is very soft and is easily applied to the skin.

Ointment Stability Tests.—The six silicone ointments previously described were tested for stability at varying temperatures. Four 10-Gm. samples of each base were placed in glass, 1-oz. ointment jars. One sample of each base was maintained at a temperature of 47°, a second at 40°, and the third and fourth samples at room temperature and 5°, respectively. For a period of one month each ointment was checked daily for signs of melting or separation. Data are shown in Table I.

Compatibility of Silicone Ointments and Official Ointments with Various Medicinals.—To determine the compatibility of silicone ointment bases with various medicinal agents commonly prescribed in ointments, a series of ointments was prepared using the six silicone bases, three U. S. P. ointments (white ointment, hydrophilic ointment, and hydrophilic petrolatum) as controls, and 17 medicinal agents. A small quantity of each medicated ointment was kept in an oven at 41–43° in order to hasten the aging process, and another sample was aged at room temperature. They were observed immediately after incorporation, in twenty-four hours, in forty-eight hours, and after a two-week period. The data presented in Table II represent the relative compatibilities as measured by the ease of mixing and by relative stabilities under the existing temperatures at specified times.

Diffusion of Iodine, Sulfathiazole, and Benzoic and Salicylic Acids from Silicone Ointment Bases and from U. S. P. Ointment Bases.—The method originated by Waud and Ramsay (16) and later used by Levy and Huyck (17) and Lockie and Sprowls (18) was used to compare the diffusion rates of iodine, sulfathiazole, and a mixture of benzoic and salicylic acids from the six silicone ointment bases and from hydrophilic ointment U. S. P., hydrophilic petrolatum U. S. P., and white ointment U. S. P.

(*a*) *Diffusion of Iodine.*—Following a modification of the procedure outlined by Lockie and Sprowls (18) an agar gel containing 4.0 Gm. of Bacto-Agar (Difco), 0.1 Gm. of soluble starch, and 100 cc. of Ringer's solution was prepared and poured into test tubes 16.5 x 150 mm. The tubes were autoclaved in the initial experiments but this was found to be unnecessary. After the agar had fully solidified, approximately 2 cc. of ointment was superimposed upon the gel. The ointment was forced into a glass tube and then a solid glass rod was used as a plunger

TABLE I.—STABILITY OF SIX SILICONE OINTMENT BASES AT VARYING TEMPERATURES[a]

Ointment Base	5° C.	Room Temp.	40° C.	47° C.
Silicone absorption base	S	S	S	4
Silicone anhydrous base	S	S	6	3
Silicone Gibson base	7	S	17	1
Silicone glyceryl monostearate base	17	S	10	1
Silicone hydrophilic ointment	S	S	S	1
Silicone cold cream	S	S[b]	4	1

[a] The numbers represent the number of days during which an ointment was stable at the specified temperature. "S" indicates that the ointment was stable during the entire test period (one month).

[b] Oil separated slightly after the test period.

TABLE II.—COMPATIBILITY OF SILICONE OINTMENT BASES AND U. S. P. OINTMENT BASES WITH VARIOUS MEDICINALS

Medicament	Silicone Absorption Base	Silicone Anhydrous Base	Silicone Gibson Base	Silicone Glyceryl Monostearate Base	Silicone Cold Cream	Silicone Hydrophilic Ointment	Hydrophilic Ointment, U. S. P.	White Ointment U. S. P.	Hydrophilic Petrolatum, U. S. P.
Aluminum Acetate Solution, U. S. P. 10%	G S S S	G O O O	G S * SW	G S SW SW SW	G SO SO O	G S S S	G S S S	G S S SO	G S S S
Ammoniated Mercury 10%	G S S S	G S S SO	G S S SW	G S SW SW SW	G O S O	G S S SW	G S S SW	G S S S	G S S S
Benzoic Acid 12%, Salicylic Acid 6%	G S S S	G S SO SO	G S S S	D S SW W	G S SO O	G S S SW	G S S SW	G S S S	G S S S
Boric Acid 10%	G S S S	G S SO O	G S S SW	W S W S	G S S SO	G S S S	G S S W	G S S SO	G S S S
Coal Tar 5% with Tween 80 5%	G S S S	G S SO O	G S S S	G S S W	G S* SO	G S S S	G S S SW	G S S S	G S S S
Ichthammol 10%	G S S SO	G S SO O	G S S S	* W	G O SO SO	G S S W	G S S W	G S S S	G S S S
Iodine Ointment, N. F., except for base	G S S S	G S O O	G S * *	G S S S	G O S O	G S SO O	G S* W	G S S S	G S S S
Iodochlorhydroxyquin 3%	D S S *	D S SO O	D S SW SW	D S S S	D S O S	D S SW SW	G S SW SW	G S S SO	G S S SO
Menthol 10%	G S S SO	G S SO O	D S SW SW	D SO O O	G S SO SO	G S S SW	G S W W	G S S S	D S S S
Methyl Salicylate 10%	G S S O	G S SO SO	G S S S	G S S S	G S SO O	G S S S	G S S W	G S S S	G S S S
Peruvian Balsam 10%	G S S S	G S SO SO	G S SW SW	G O S O	G S S WO	G S S S	G S S *	G S S S	G S S SO
Phenol, Liquefied 2%	G S S S	G S SO SO	G S S WO	G S S W	G S S WO	G S S W	G S S W	G S S S	G S S S
Resorcinol 1%	G S S S	G S SO SO	G S S W	G S S S	G S S O	G S S W	G S S W	G S S S	G S S S
Sulfathiazole 5%	G S S S	G S SO SO	G S S SW	G S S S	G S SO SO	G S S SW	G S S SW	G S S S	G S S S
Sulfur 10%	G S S S	G S SO SO	G S S S	G S S S	G S SO SO	G S S SW	G S S SW	G S S S	G S S SO
Zinc Oxide 10%	G S S S	G S SO O	G S SW S	D * W	G S SO O	G S S W	G S S W	G S S SO	G S S SO
			W W	SW SW W	S O S O	W W W	S W W	* 0	SO W W

1a 2a 3a 4a
2b 3b 4b
Various times and temperature are indicated by position:
1a—Compatibility at time of mixing, room temperature
2a—Room temperature, 24-hour period.
3a—Room temperature, 48-hour period.
4a—Room temperature, 2-week period.
2b—41–43° C., 24-hour period.
3b—41–43° C., 48-hour period.
4b—41–43° C., 2-week period.

G—Mixed well.
D—Difficulty in mixing.
S—Stable.
O—Oil separated from base.
W—Water separated from base.
SW—Water separated slightly.
SO—Oil separated slightly.
WO—Water phase and oil phase separated.
*—Possibly very slight separation.

to eject the ointment in a uniform layer upon the agar gel. It was found that the medicinal agent diffused at the same rate whether 1 cc. or 2 cc. of ointment was used in the test. The ointments corresponded to iodine ointment N. F. (19) except for their bases. Four per cent iodine, 4% potassium iodide, and 12% glycerin were incorporated with 80% of the particular base. The diffusion rate was determined by measuring the width of the purple band formed by the starch and iodine reaction at intervals of one, three, six, nine, eighteen, and twenty-four hours for the first day, and every twenty-four hours thereafter for one week. An aqueous solution containing 4% iodine and 4% potassium iodide was superimposed upon a starch-agar gel to serve as a control.

The tests were conducted in triplicate and the average width of diffusion in centimeters was plotted against time to determine the diffusion rates, as shown in Fig. 1. Silicone Gibson base showed the greatest rate of iodine diffusion and hydrophilic ointment U. S. P., silicone glyceryl monostearate base, silicone anhydrous base, silicone hydrophilic ointment, silicone cold cream, silicone absorption base, hydrophilic petrolatum U. S. P., and white ointment U. S. P. followed in that order.

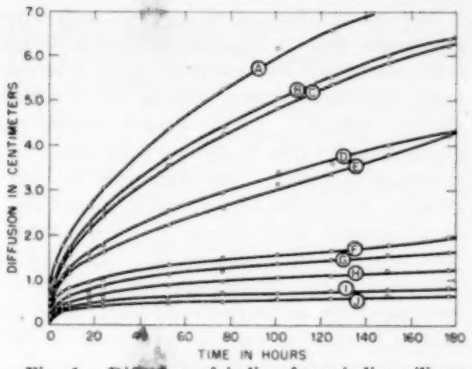


Fig. 1.—Diffusion of iodine from iodine silicone ointments and from iodine ointments prepared with U. S. P. ointment bases. *A*, solution; *B*, silicone Gibson base; *C*, hydrophilic ointment U. S. P.; *D*, silicone glyceryl monostearate base; *E*, silicone anhydrous base; *F*, silicone hydrophilic ointment; *G*, silicone cold cream; *H*, silicone absorption base; *I*, hydrophilic petrolatum U. S. P.; *J*, white ointment U. S. P.

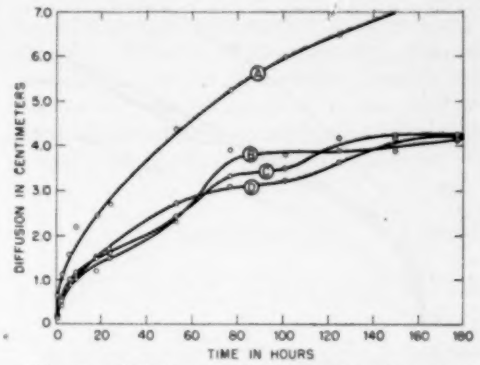


Fig. 2.—Diffusion of sulfathiazole from 5 per cent sulfathiazole silicone ointments and from 5 per cent sulfathiazole ointments prepared with U. S. P. ointment bases. *A*, solution; *B*, hydrophilic ointment U. S. P.; *C*, silicone glyceryl monostearate base; *D*, silicone cold cream.

(*b*) *Diffusion of Sulfathiazole.*—Five per cent sulfathiazole was added to the same nine ointment bases, and the diffusion rate of the drug was studied by superimposing the ointment over an agar gel containing Ehrlich's reagent (17). Ehrlich's reagent gives a bright yellow color with sulfathiazole, and the width of the yellow band formed in the gel was measured in the same manner as was the purple band in the iodine test. Lockie and Sprowls used 1.0 cc. of this reagent to 100 cc. of Ringer's solution in preparing the agar gel. This gel was found to produce only a faint color when a sulfathiazole ointment was superimposed upon it. Therefore a gel containing nearly 5% of Ehrlich's reagent was prepared in accordance with the method of Levy and Huyck (17). An aqueous solution (5%) of sodium sulfathiazole was used as a control. Results are shown in Figs. 2 and 3. The diffusion of sulfathiazole from silicone absorption base, white ointment U. S. P., and hydrophilic petrolatum U. S. P. was too slight for measurement. The diffusion rates of sulfathiazole from the other six bases were nearly the same; however, silicone hydrophilic ointment exhibited a slightly greater rate of sulfathiazole diffusion than did the others.

(*c*) *Diffusion of Benzoic and Salicylic Acids.*—Whitfield's ointment was the third ointment selected for diffusion tests. A gel consisting of 100 cc. of Ringer's solution, 4.0 Gm. of Bacto-Agar (Difco), and 0.2 cc. of ferric chloride solution N. F. was prepared, and benzoic acid and salicylic acid (12% and 6%, respectively) were incorporated into the nine different ointment bases. The procedure for conducting this test was the same as that for iodine and sulfathiazole. The diffusion zone was identified by its well-defined, violet color. In the first experiment, diffusion readings were consistent until the fifty-three-hour period. At that reading, it was noticed that the violet color was beginning to fade in some of the tubes. The readings at seventy-seven hours were widely varied, and the last reading was taken at the end of 101 hours. The experiment was repeated and the tubes were kept in the dark. The last two readings, at 148 hours and 172 hours, do not check too closely, but they serve as an indication of the diffusion rates. An aqueous solution containing 12% sodium benzoate and 6% sodium salicylate was used as a control. Results are shown in Figs. 4 and 5. Silicone anhydrous base and silicone glyceryl monostearate base had a slightly greater diffusion rate than did the next five ointments—silicone cold cream, silicone absorption base, silicone Gibson base, silicone hydrophilic ointment, and hydrophilic ointment U. S. P.; white ointment U. S. P. and hydrophilic petrolatum U. S. P. showed the lowest diffusion rates.

Silicone Oils in Liniments.—Silicone oils present two obvious advantages over vegetable oils as vehicles or ingredients of vehicles for liniments: they are odorless and they do not become rancid. Camphor liniment U. S. P.; ammonia liniment N. F.; and ammonia and camphor liniment, anodyne liniment, belladonna and chloroform liniment, and

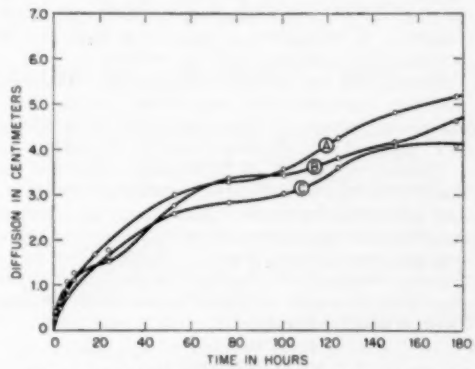


Fig. 3.—Diffusion of sulfathiazole from 5 per cent sulfathiazole silicone ointments and from 5 per cent sulfathiazole ointments prepared with U. S. P. ointment bases. *A*, silicone hydrophilic ointment; *B*, silicone Gibson base; *C*, silicone anhydrous base.

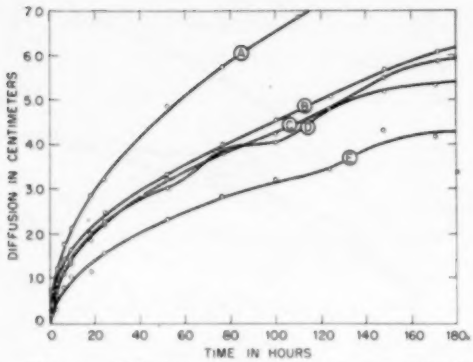


Fig. 4.—Diffusion of benzoic acid and salicylic acid from ointments prepared with silicone ointment bases and from ointments prepared with U. S. P. ointment bases. *A*, solution; *B*, silicone glyceryl monostearate base; *C*, silicone cold cream; *D*, hydrophilic ointment U. S. P.; *E*, white ointment U. S. P.

Pusey's calamine and zinc oxide liniment from "The Pharmaceutical Recipe Book," 3rd ed. (20) were prepared and compared with the same liniments made by substituting 3-cts. silicone oil for the official liniment base.

Silicone oil was used in place of the cottonseed oil in camphor liniment. Aside from the fact that the silicone liniment had a more pleasing odor and was less viscous, there was little difference between the silicone camphor liniment and the official preparation.

Silicone ammonia liniment compared well with the National Formulary liniment because 1% oleic acid is included in the formula. The oleic acid forms a soap with the diluted ammonia solution, and the silicone oil is emulsified to produce a stable, viscous preparation. In "The Pharmaceutical Recipe Book" formula for ammonia and camphor liniment, oleic acid is not included; therefore a satisfactory emulsion liniment was not obtained.

The problem of including aqueous solutions in silicone liniments can be easily overcome by the addition of an emulsifying agent to the liniment formula. G Emulsifier, a soap, or a Span-Tween combination is suggested for this purpose.

Silicone oils will not mix with alcoholic solutions, and this incompatibility was shown in the two-layered appearance of silicone belladonna and chloroform liniment and of silicone anodyne liniment.

Silicone oil proved to be a good substitute for liquid petrolatum in Pusey's petrolatum liniment, and the remaining three silicone liniments—silicone camphor and turpentine liniment, silicone menthol liniment, and silicone Pusey's calamine zinc oxide liniment—were pharmaceutically as good as or better than liniments made with olive oil as required by the "Recipe Book" formula.

SUMMARY

The value of silicone oils as dermatological vehicles was studied by determining their pharmaceutical properties.

The compatibility of silicone oils with other substances used in formulating dermatological bases was tested by incorporating 42 other vehicles with each of three silicone oils of widely varying viscosity. Cetyl alcohol, theobroma oil, diglycol stearate S, glyceryl monosteate, hydroxystearin sulfate, lauric acid, wool fat, polyethylene glycol 400 monostearate, stearic acid, stearyl alcohol, and Unibase produced good binary mixtures with silicone oils. Mixtures of silicones with carbowaxes, soft soap, and glycerin were not stable, and waxes had a tendency to appear as granules in wax-silicone mixtures. Silicone oils were practically immiscible with vegetable oils, liquid petrolatum, some of the acids, and some of the alcohols, including cholesterol.

A series of emulsifying agents for silicone oils was investigated. The most satisfactory emulsifier of the group was a combination of Atlas G-2125 and Atlas G-2126. Several other emulsifying agents produced good, stable emulsions of the dimethylsiloxane polymers and water.

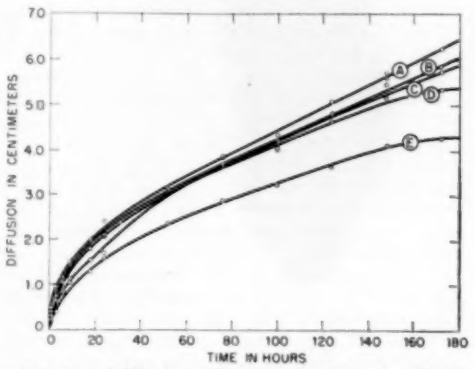


Fig. 5.—Diffusion of benzoic acid and salicylic acid from ointments prepared with silicone ointment bases and from ointments prepared with U. S. P. ointment bases. *A*, silicone anhydrous base; *B*, silicone Gibson base; *C*, silicone absorption base; *D*, silicone hydrophilic ointment; *E* hydrophilic petrolatum U. S. P.

Six silicone oil-ointment bases—silicone hydrophilic ointment, silicone cold cream, silicone anhydrous base, silicone absorption base, silicone Gibson base, and silicone glyceryl monosteate base—were devised. The physical properties of these ointments were described, and their stabilities at four different temperatures were determined.

The same six bases and three official ointments were tested for compatibility with 17 medicinals commonly prescribed in ointments. The silicone oil-ointment bases compared favorably with

the official ointments used as controls in both ease of mixing and in the stability of the medicated ointments.

The rates of diffusion of iodine, sulfathiazole, and a mixture of benzoic and salicylic acids from the six silicone bases were compared with each other and with the rates of diffusion from three U. S. P. ointments. Silicone Gibson base showed the greatest rate of iodine diffusion, and hydrophilic ointment U. S. P., silicone glyceryl monostearate base, silicone anhydrous base, silicone hydrophilic ointment, silicone cold cream, silicone absorption base, hydrophilic petrolatum U. S. P., and white ointment U. S. P. followed in that order. The diffusion of sulfathiazole from silicone absorption base, white ointment U. S. P., and hydrophilic petrolatum U. S. P. was too slight for measurement. The diffusion rates of sulfathiazole from the other six bases were nearly the same. In the diffusion of the benzoic acid-salicylic acid mixture, silicone anhydrous base and silicone glyceryl monostearate base had a slightly greater diffusion rate, and white ointment U. S. P. and hydrophilic petrolatum U. S. P. showed a lower diffusion rate than did the other five bases.

The use of silicone oils as vehicles or as ingredients of vehicles for liniments was studied by substituting silicone oils for the liquid petrolatum and vegetable oils prescribed as liniment bases by the official and "Pharmaceutical Recipe Book" formulas. Silicone oil liniments were pharmaceutically similar to the accepted formulas and silicone oils were advantageous in that they are odorless, not subject to rancidity, and their wide viscosity range allows variation in liniment viscosity.

REFERENCES

(1) Rochow, E. G., "An Introduction to the Chemistry of the Silicones," 2nd ed., John Wiley & Sons, Inc., New York, 1951.
(2) "The Silicones—Cornerstone of a New Industry," *Fortune*, **35**, No. 5, 104(1947).
(3) Jaques, L. B., Fidlar, E., Felsted, E. I., and MacDonald, A. G., *Can. Med. Assoc. J.*, **55**, 26(1949).
(4) Marguilies, H., and Barker, N. W., *Am. J. Med. Sci.*, **218**, 42(1949).
(5) Marguilies, H., and Barker, N., *ibid.*, **218**, 52(1949).
(6) Goldman, R. (to Premo Pharmaceutical Laboratories, Inc.), U. S. pat. 2,504,482, April 18, 1950, *Chem. Abstr.*, **44**, 5552e(1950).
(7) Yen, E. C., and Stirn, F. E., U. S. pat. 2,512,912, *Chem. Abstr.*, **44**, 8067a(1950).
(8) *Dow Corning Silicone Note Book*, Dow Corning Corp., Midland, Mich., September, 1948.
(9) Rowe, V. K., Spencer, H. C., and Bass, S. L., *J. Ind. Hyg. Toxicol.*, **30**, 332(1948).
(10) Kern, S. F., Anderson, R. C., and Harris, P. N., THIS JOURNAL, **38**, 575(1949).
(11) Bolton, B. A., Atlas Powder Co., Wilmington, Del., private communication, April 11, 1951.
(12) "United States Pharmacopeia," Fourteenth Revision, Mack Publishing Co., Easton, Pa., 1950.
(13) Ehrenstein, E., *Am. J. Pharm.*, **122**, 208(1950).
(14) Gibson, A. J., Parker, H. E., and Almus, A., THIS JOURNAL, **30**, 196(1941).
(15) Goodman, H., *Am. Profess. Pharmacist*, **8**, 355(1942).
(16) Waud, R. A., and Ramsay, A., *Can. Med. Assoc. J.*, **48**, 121(1943).
(17) Levy, B., and Huyck, C., THIS JOURNAL, **38**, 611(1949).
(18) Lockie, L. D., and Sprowls, J. B., *ibid.*, **38**, 222(1949).
(19) "The National Formulary," 9th ed., Mack Publishing Co., Easton, Pa.
(20) "The Pharmaceutical Recipe Book," 3rd ed., Mack Publishing Co., Easton, Pa., 1943.

The Synthesis of Carbon-14 Labeled Chloroform*,†

By HENRY C. REITZ, FREDERICK J. CARLETON,‡ and JOHN E. CHRISTIAN

A procedure is described for the synthesis of carbon-14 labeled chloroform, starting with $BaC^{14}O_3$ and involving the preparation of barium carbide, acetylene, acetaldehyde, and finally chloroform in an over-all yield of 36%.

THE synthesis of labeled chloroform has not been previously reported in the literature. Cramer and Kistiakowsky (1) have succeeded in reducing labeled barium carbonate to the carbide. The preparation of acetylene on a micro scale was performed by Arrol and Glascock (2). Vennesland, *et al.* (3), described the transformation of acetylene to acetaldehyde. The synthesis of chloroform from acetone by use of HTH is described by Baril (4).

After careful consideration of various syntheses of chloroform reported in the literature, the following series of reactions was employed for the synthesis of carbon-14 labeled chloroform:

$$2BaC^*O_3 + {}^*5Mg \rightarrow BaC^*_2 + BaO + 5MgO$$
$$BaC^*_2 + 2H_2O \rightarrow Ba(OH)_2 + HC^*{\equiv}C^*H$$
$$HC^*{\equiv}C^*H + H_2O \rightarrow {}^1/_2C^*H_3CHO + {}^1/_2CH_3C^*HO$$

* Received August 22, 1952, from the Chemistry Department and the School of Pharmacy, Purdue University, Lafayette, Ind.
Presented to the Scientific Section, A. PH. A., August, 1952.
† The isotopes used in this research were granted on allocation from the United States Atomic Energy Commission.
‡ Present address: National Dairy, Research Laboratories, Inc., Oakdale, Long Island, N. Y.

$$C^*H_3CHO + CH_3C^*HO + 3Ca(OCl)_2 \rightarrow C^*HCl_3 + CHCl_3 + 2Ca(OH)_2 + {}^1/_2Ca(HC^*OO)_2 + {}^1/_2Ca(HCOO)_2$$

EXPERIMENTAL

A quantity of 1 Gm. of barium carbonate is mixed intimately with 400 mg. of impalpable magnesium powder and 50 mg. of silicon dioxide in the apparatus shown in Fig. 1. The mixture is then covered with an additional 250 mg. of the magnesium powder. A two-hole rubber stopper containing two electrodes sealed in glass tubes is inserted in the Carius tube. Parr Bomb fuse wire is connected to the two electrodes. Experimentation has shown that the best results in starting the reaction are obtained if the wire extends just slightly below the layer of magnesium powder. A potential of 60 volts applied

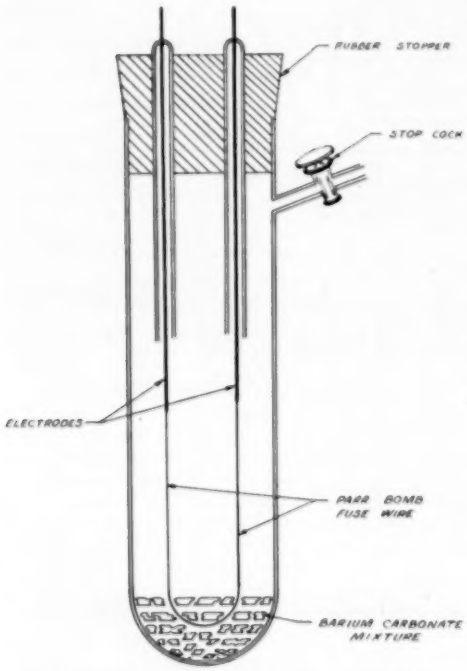


Figure 1.

across the electrodes fires the fuse and starts the reaction which requires only a few seconds to go to completion. Although an exothermic flash reaction occurs, high pressures do not develop in the bomb. It is advisable to use tubing 3 mm. in diameter since thinner walled tubing does not resist the high temperature of the reaction.

The use of quantities in excess of 2 Gm. has caused a number of tubes to crack. The mixture described has been successful in more than sixty reactions. The tube should be annealed prior to using to overcome possible strains.

After the tube has cooled, the rubber stopper is removed and another stopper fitted with a separatory funnel and an outlet tube is substituted (Fig. 2). For handling larger quantities of chloroform, two additional 1-Gm. portions of barium carbonate are reduced to the carbide in separate combustion tubes and added to the original tube.

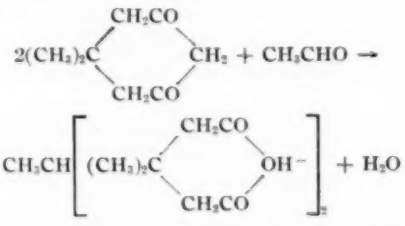
A catalytic solution is prepared by dissolving 2 Gm. of mercuric sulfate and 6 Gm. of concentrated sulfuric acid in water to a total volume of 100 ml. The catalytic solution (10 ml.) in the form of frozen rods is then placed in a Carius tube previously prepared for the condensation of acetylene. If the catalytic solution is introduced into the Carius tube and then frozen in the liquid nitrogen bath, the tube cracks when removed from the bath. A simple method to obtain the frozen rods is to pour the catalytic solution into a 10-ml. tube sealed at one end and to place the tube in a liquid nitrogen bath. The catalytic solution is frozen within a minute. When the sealed end of the tube is broken, the solidified rods can easily be removed.

The Carius condensing tube is connected by rubber tubing to a vapor trap connected in turn to the combustion tube (Fig. 2).

The system is flushed with helium and evacuated to a pressure of 4 mm. of mercury. The acetylene is generated by slowly adding 20 ml. of water to the carbide. After the initial frothing ceases, the tube is heated intermittently for twenty minutes to assure complete generation. Any water vapor formed is collected in the water trap.

The stopcock between the condensation tube and the water trap is closed and the condensing unit is disconnected. The condensation tube is allowed to come to room temperature, placed in a Carius furnace, and heated for five minutes at 100°. The tube is then removed from the furnace and allowed to cool to room temperature; the acetaldehyde is then distilled. A circulating pump is used to force ice-salt brine through the condenser. The acetaldehyde-water mixture is collected in a tube cooled by ice-salt brine.

In determining the amount of acetaldehyde obtained in a number of runs using unlabeled barium carbonate, the aldehyde was precipitated as a methone (5,5 - dimethyl - 1,3 - cyclohexanedione) derivative which is prepared in the following manner: 0.4 Gm. of methone, 4 ml. of 95% ethanol, and one drop of piperidine are added to the acetaldehyde. The mixture is gently refluxed for five minutes, and water is added until the cloud point appears. The derivative is separated by filtration, washed with 2 ml. of cold 50% ethanol, dried, and weighed. A yield of 62%, based on the carbonate, was obtained. The reaction involved is as follows:



Eight grams of acetaldehyde C. P. is added to the acetaldehyde obtained by the distillation, and the combined portions are added to a HTH solution contained in a 1-L. Claissen flask attached to a condenser. The HTH solution is prepared by grinding

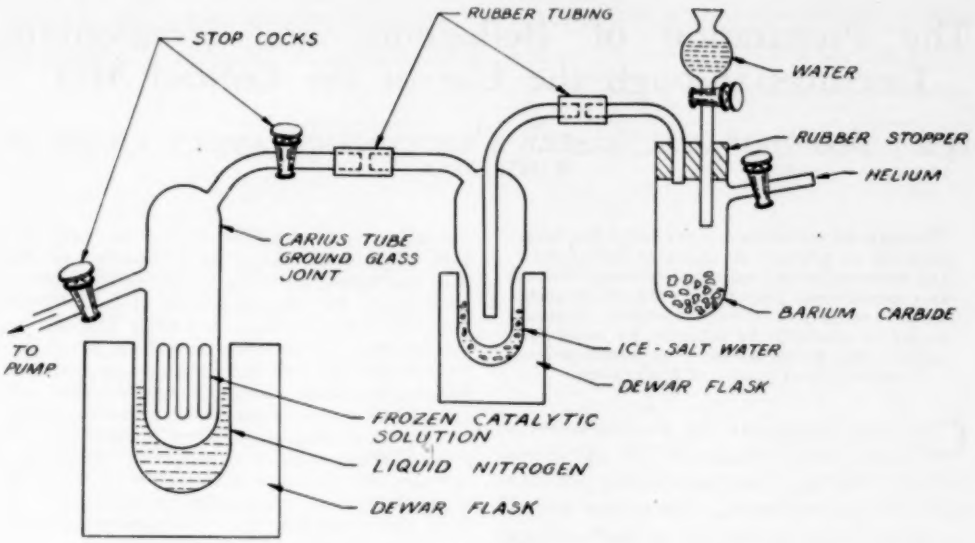


Figure 2.

100 Gm. of HTH[1] powder and 0.5 Gm. of sodium hydroxide with 250 ml. of water and decanting the solution into the Claissen flask. The reaction is immediate and accompanied with a great deal of frothing. The fraction distilling at 55–63° (n_D^{20}, 1.4452) is collected in a separatory funnel and cooled with ice water, and the chloroform layer is removed. A yield of 59%, based on the acetaldehyde, was obtained.

For the preparation of chloroform labeled with carbon-14, precisely the same procedure is followed except that carbon-14 labeled $BaCO_3$ with the desired activity is added to the unlabeled carbonate. Synthesis using labeled carbonate was accomplished after mixing 1 mc. of $BaC^{14}O_3$ (isotopic ratio, 3.27%; 86.2 mg.) with the barium carbonate in the initial reaction. Yields were comparable to those obtained with the nonradioactive syntheses.

The specific activity of the product was determined by oxidizing a sample of the chloroform (120.5 mg.) and collecting the evolved CO_2 in 0.25 *N* $Ba(OH)_2$. The BaC^*O_3 was collected on filter paper (2.54 cm.^{2}) by gentle suction, followed by washing with water and alcohol. The filter was removed, set on a dish on which was placed a thin film of collodion to prevent curling, and dried in a vacuum oven at 60° for one hour. The sample was then counted in a windowless gas flow counter (Q-gas) and the activity was compared with that obtained from a National Bureau of Standards carbon-14 sample under identical conditions. The specific activity was found to be 0.002 microcurie/mg.

[1] High-test calcium hypochlorite.

SUMMARY

1. A method for the synthesis of carbon-14 labeled chloroform, starting with $BaCO_3$, is described in detail.

2. A yield of 62 per cent of acetaldehyde based on the barium carbonate and a yield of 59 per cent of chloroform based on the acetaldehyde were obtained.

3. Starting with 1 mc. of $BaC^{14}O_3$, a 36 per cent over-all yield (based on carbonate) of chloroform with a specific activity of 0.002 microcuries/mg. was obtained.

REFERENCES

(1) Cramer, R. D., and Kistiakowsky, G. B., *J. Biol. Chem.*, **137**, 549(1941).
(2) Arrol, W. J., and Glascock, R., *Nature*, **159**, 810(1947).
(3) Vennesland, B., Solomon, A. K., Buchanan, J. M., Cramer, R. D., and Hastings, A. B., *J. Biol. Chem.*, **142**, 371 (1942).
(4) Baril, O. L., *J. Chem. Educ.*, **17**, 565(1940)

The Preparation of Belladonna and Stramonium Tinctures through the Use of the Colloid Mill*

By S. J. DEAN, DONALD C. BRODIE, E. BROCHMANN-HANSSEN, and SIDNEY RIEGELMAN†

Through the use of the colloid mill it has been possible to prepare tinctures of belladonna and stramonium in a matter of minutes, whereas conventional extraction methods require periods ranging from days to weeks. A great saving of time may be achieved by using the colloid mill either alone or in combination with a short period of maceration.

CLASSICAL procedures for the extraction of crude drugs preparatory to the manufacture of tinctures and fluidextracts are laborious and time-consuming. Hence, any method by which the time of manufacture can be reduced significantly is of industrial and academic interest. This paper reports the application of modern pharmaceutical milling equipment to the preparation of tinctures of solanaceous drugs by which the time of manufacture (exclusive of assay) can be reduced to a matter of minutes.

Two general procedures, namely, maceration and percolation, have long been the basis for the removal of medicinal plant constituents. The U. S. P. (1) and N. F. (2) methods for extraction are based on these methods. Modifications of these procedures have been necessary from time to time because of physical and chemical properties of the crude drugs. Among the modifications for which there has been some application are: diacolation (3), mulcolation (4), and evacolation (5). These are pressure or vacuum methods of extraction or both. Pressure extraction similar to that used in the wine industry has been applied to pharmaceutical systems but with success only in those cases which employ nonalcoholic menstrua (6). The most recent extractive procedures have been the use of the Waring blendor by Bay and Gisvold (7) and King and Gisvold (8) and the pressure cooker by Greco and Du Mez (9).

EXPERIMENTAL

Samples of belladonna leaf U. S. P. and stramonium N. F. were reduced to a No. 40 (or less) powder in a Fitzpatrick Homoloid machine. Four series of experiments were performed, using 100 Gm. of powdered drug and 1,000 cc. of official menstruum for either the corresponding U. S. P. or N. F. tincture. The experiments were as follows: (*a*) the drug was suspended in the menstruum and passed immediately through a Charlotte Model A colloid mill,[1] which was adjusted for a single passage; (*b*) the drug was allowed to macerate in the menstruum for five, fifteen, and thirty minutes, respectively, followed by a single passage through the colloid mill; (*c*) the mill was adjusted for recirculation and the suspension of drug was allowed to recirculate for five and fifteen minutes, respectively; (*d*) tinctures were prepared by maceration for five, twenty, and thirty-five minutes without the use of the mill. In each experiment the marc was removed immediately by centrifuging (four minutes at 1,500 r. p. m.) and the tinctures so obtained were assayed for alkaloid content according to U. S. P. XIII procedures. The completeness of extraction was calculated on the basis of the alkaloid content of the crude drug as determined by the official assay procedures. The results of the alkaloid determinations are found in Table I.

DISCUSSION

The object of any extraction process is the removal of the desired plant or animal constituent through the use of a solvent that will be indifferent to the extraneous plant or animal solids. The essential steps in extraction are: (*a*) wetting and penetration of the cell wall; (*b*) contact between the solvent and the cellular constituents with subsequent dissolution or elution with the solvent; and (*c*) the exchange of intracellular solution with the extracellular solvent.

In extraction procedures, surface tension, diffusion, hydrostatic pressure, adhesion, capillarity, and convection currents are physical forces of considerable importance. However, the rate at which the active constituent is removed finally depends upon the permeability of the cell wall. This factor will control the over-all rate of exchange of the solution of the plant constituents with the pure solvent.

The design of the percolating chamber and the degree of packing of the contents assure maximum time of contact between the cell and the solvent, but it is doubtful if either of these factors modify the cell permeability except in a minor way. Extraction studies of the past have been devoted to modifications of the classical maceration and percolation procedures. It seems unlikely that continued study of conventional gravitational methods will lead to an increase in the amount extracted or the rate at which this is accomplished. It seems reasonable to assume, however, that any procedure in which cell permeability is increased significantly will enhance the rate of extraction.

* Received May 5, 1950, from the University of California College of Pharmacy, The Medical Center, San Francisco.
Presented to the Scientific Section, A. PH. A., Atlantic City meeting, May, 1950.
† The authors wish to acknowledge the assistance of Professor Robertson Pratt in examining the marc.

[1] Rotor velocity, 3,600 r. p. m.; length of rotor-stator, 6.5 cm.; medial circumference, 16.5 cm.; spacing, 0.0125 cm.

TABLE I.—BELLADONNA AND STRAMONIUM TINCTURES PREPARED BY MACERATION AND COLLOID MILL PROCEDURES

Extraction Procedure	Alkaloid Extracted, Mg./100 Cc.[a]		Alkaloid Recovered, % Based on Soxhlet Extraction		Total Extractive, Belladonna Only, Gm./100 Cc.	
	Belladonna	Stramonium	Stramonium	Belladonna	Recovered Tinct.	Adjusted Tinc. (Alkaloid, 30 Mg./100 Cc.)
Colloid mill, once through	26.4	21.6	70.4	62.9	1.64	1.86
5-min. maceration, and once through mill	34.5	.....	...	82.2	1.85	1.61
15-min. maceration, and once through mill	38.3	26.7	86.6	91.2	1.81	1.42
30-min. maceration, and once through mill	39.8	29.8	97.1	94.8	1.85	1.39
5-min. maceration	15.6	.....	...	37.2	1.44	2.77
20-min. maceration	31.2	25.3	82.4	74.3	1.82	1.75
35-min. maceration	36.0	26.7	87.0	85.7	1.90	1.58
5-min. recirculation through colloid mill	41.8	27.3	88.9	99.5	..	...
15-min. recirculation through colloid mill	42.2	28.8	93.8	100.5	1.94	1.38
Soxhlet extraction	42.0 Mg./10 Gm.	30.7 Mg./10 Gm.	100.0	100.0	...	...

[a] Average values of two to four determinations.

When a suspension of a powdered drug in a liter of the appropriate solvent was passed through the colloid mill, an amount of alkaloid that requires several days by percolation was obtained in a matter of minutes. The mill used in these studies is of such specifications that the shearing forces are applied to a volume of approximately 1.3 cc. Furthermore, this volume of suspension is subjected to the milling forces for approximately 0.04 second. Even with such a short period of contact between the drug and the mill, the amount of alkaloid extracted compares favorably with the amount removed by classical procedures.

The data listed in Table I reveal that the colloid mill enhances extraction over and above that resulting from a simple maceration procedure. In the case of belladonna, five minutes' maceration gave a yield of only 37.2% of alkaloids, whereas a single passage through the mill (actually a shorter time of contact between drug and solvent) resulted in 62.9% recovery of the alkaloid and also a greater total extractive. The effect of the colloid mill becomes even more apparent when the results obtained by maceration are compared with those obtained by recirculation through the colloid mill. In this case the 37.2% recovery is increased to 99.5% when the drug is milled continually for five minutes rather than subjected to maceration for the same period of time. If the results from maceration alone are compared with those obtained by the colloid mill in conjunction with maceration, the same enhancement due to the mill can be recognized.

The results obtained with stramonium agree fairly well with those obtained with belladonna, although there are certain differences. Thus, the very high recoveries of approximately 100% obtained by recirculation of belladonna could not be duplicated for stramonium. More experimental evidence is needed, however, before any conclusions can be drawn from these observations.

The amounts of tincture recovered by the colloid mill process from the original 1,000 cc. of menstruum ranged from 800 to 850 cc. There were no detectable differences in either the color or the taste of the tinctures prepared by the colloid mill method when compared with those prepared by the official procedures.

The experiments for total extractive on the belladonna tinctures suggested a positive correlation between alkaloid recovery and the extractive. When the drug was macerated for five minutes, the extractive was 1.44 Gm./100 cc. (37.2% recovered alkaloid), whereas the same time for a single passage through the mill yielded 1.64 Gm./100 cc. of extractive (62.9% recovered alkaloid). When the amount of alkaloid recovered was about 85% or greater, the amount of extractive remained relatively constant, varying from about 1.85 to 1.94 Gm./100 cc., the latter value corresponding to 100% recovery of alkaloid. The results of these experiments are probably of more academic than practical interest because of the inverse relationship between alkaloid content and total extractive that results when the preparations are adjusted to U. S. P. specifications.

A microscopic examination was made of the marc obtained from a tincture prepared by the colloid mill method, but it revealed only an insignificant degree of damaged or ruptured cells. The enhancement of the extraction by the mill, therefore, does not seem to be due to an increased exposure of cell content, at least not to any great extent. Undoubtedly the complexity of the streaming forces with which the drug comes in contact brings about a modification of those factors concerned with extraction, and it seems reasonable to believe that cellular permeability may undergo considerable alteration.

SUMMARY

The possibility of preparing tinctures of solanaceous drugs by passing a suspension of the powdered drug in appropriate menstruum through a colloid mill has been investigated. The results indicate that the colloid mill is capable of enhancing extraction to a considerable degree and that a

great saving of time may be achieved in the preparation of these products by using the colloid mill, either alone or in combination with a short period of maceration.

REFERENCES

(1) "United States Pharmacopeia," Thirteenth Revision, Mack Publishing Co., Easton, Pa., 1947, p. 708.
(2) "The National Formulary," 8th ed., Mack Publishing Co., Easton, Pa., 1946, p. 758.
(3) Breddin, H., *Pharm. Zig.*, **79**, 148, 163(1934); through *Chem. Abstr.*, **28**, 2465(1934); U. S. pat. 2,046,055 (1936).
(4) Kummer, R., *ibid.*, **79**, 664(1934); through *Chem. Abstr.*, **28**, 5176(1934).
(5) Kessler, E., *ibid.*, **80**, 1080(1935); through *Chem. Abstr.*, **30**, 569(1936).
(6) Anon., *Drug & Cosmetic Ind.*, **48**, 207(1941).
(7) Bay, G., and Gisvold, O., THIS JOURNAL, **37**, 314 (1948).
(8) King, R. E., and Gisvold, O., *ibid.*, **39**, 109(1950).
(9) Greco, S. J., and Du Mez, A. G., *ibid.*, **39**, 560(1950).

A Study of Suppository Bases. I. Review of Literature*,†

By HERBERT MICHEAL GROSS and CHARLES H. BECKER‡

A review of the literature on suppository bases shows that much work has been done on fat-type bases such as cocoa butter. Some investigations have been made on water-soluble and emulsion type bases. There seems to be little or no work done with such compounds as plastics, synthetic resins, and other similar condensation products. The review on suppository bases includes 108 references, containing data in classified form.

ALTHOUGH suppositories are not too popular a mode of administering drugs, this dosage form will always have its place in medicine. Actually, the field needs further investigation, and improvement in the stability, liberation of drug, and advantageous therapeutic action of this dosage form will enhance its importance.

By definition (1), suppositories are easily fusible preparations usually in the form of cones or cylinders for introduction into the rectum, vagina, etc.; pharmaceutically, the term connotes preparations of solid form for introduction into any orifice of the body except the mouth. From a more restricted viewpoint, they are interpreted to mean products intended for insertion into the rectum, vagina, and urethra, whereas those used in nose or ear are termed "bougies." In modern medicine, suppositories are chiefly used via rectum and vagina.

Suppositories are an ancient dosage form. References were made to them in Papyrus Ebers dating about 1500 B.C. (2) and in the works of Hippocrates. Despite its early adoption the form does not enjoy popular usage even today because of its unesthetic quality.

From the original concepts of suppositories as clyster, enematic, and evacuant, their usage has broadened and become widespread. Sollman (3) states that the suppository can be used locally where ointments cannot be employed. Davison (4) cites its use as a restroperistaltic. While the primary use of suppositories is for their systemic action, Caspari and Kelly (5) mention that this form insures a slow, uniform diffusion of medicament to the internal parts to which it is applied, while other articles (6) discuss the absorption or resorption of medicaments for systemic use.

In an anonymous article (6) from France and two papers (7, 8) from Great Britain, the physiological aspects of absorption from the rectum and vagina are discussed. The suppository presents an ideal mode of administration of water-soluble drugs when a prompt reaction is desired. The lower parasphincteral region of the rectum, with its numerous anastomotic vessels, affords an absorptive surface from which soluble substances rapidly pass directly into venous circulation. This action is almost as rapid as by venous injection, since absorption is directly into the blood stream. In contrast, intramuscular injection must rely on lymphatic absorption for its action. In addition to systemic effect, contraceptive suppositories are particularly suited to vaginal administration because of their convenience (9).

Suppositories are of advantage in instances where oral administration is impossible due to the taste of the drug or when the drug causes gastric disturbances, nausea, and vomiting (10),

* Received July 31, 1952, from the College of Pharmacy, University of Florida, Gainesville.
† Abstracted in part from a dissertation to be presented to the Graduate Council of the University of Florida by Herbert Micheal Gross in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
‡ Associate Professor of Pharmacy, College of Pharmacy, University of Florida.

particularly in the case of children who refuse unpalatable medication or persons who cannot swallow pills or tablets.

The efficacy of the medicament incorporated into the suppository is dependent upon the base used (11); the rate of diffusion is related to the vehicle employed. Regardless of this primary factor, the drug must itself be finely subdivided, evenly distributed in the base, and available for absorption from it. Husa (12) remarks that the drug must be in readily absorbable form and should come into close contact with the mucosa to produce a systemic effect.

Buchi and Oesch (13) stated that for best absorption of the drug from its vehicle, it should be incorporated into some of the melted base or be soluble in the base. They further mentioned that solvents such as alcohol are to be avoided since they cause irritation of the mucous membranes. Another means by which absorption may be aided is emulsification of the drug, particularly in an oil-in-water base.

For local action, the systemic effect is not important; rather, prolonged intimate contact of the medicament with the area treated is desired. Incorporation of local anesthetics and antiseptics for hemorrhoids and vaginal infections and the use of glycerol suppositories to effect prompt evacuation are examples of such local action.

The practice of using dusting powder to prevent suppositories from sticking to each other or to the mold should be avoided, since, as McCormick and Ramsey (14) stated, nonhealing granulomatous lesions may develop from repeated use of such products.

In order that a drug may be utilized from the base, the suppository must be retained in the orifice into which it is introduced. Melting which results in rectal leakage is very undesirable and the expulsion of the suppository by contraction of the anal sphincters must be avoided. The former difficulty is rectified by proper selection of the base, while the latter problem is solved by use of a streamlined suppository form where the greatest diameter is about one-fourth the distance from the tip and gradually decreases toward the base (11). Rectal contractions cause this form to move upward in the rectum, obviating the possibility of expulsion.

Another difficulty is the defecation reflex stimulated by the presence of any solid body in the rectum. Therefore, unless a suppository dissolves or melts quickly after insertion, a strong reflex is perpetrated, evacuation results, and the medication is ineffective.

Rectal suppositories are usually bullet-shaped and tapered at one end, while vaginal suppositories (pessaries) may be globular or ovoid in shape. Specifications of these suppository types are mentioned in U. S. P. XIV.

CLASSIFICATION

There are four classifications into which suppository bases may be divided. The first is the fat or oil type whose action depends upon melting at body temperature to release medication. The second is the glycero-gelatin base suppository which absorbs water, dissolves, and releases the water-soluble medicament. The third includes the water-soluble or water-miscible polymers, glycol substitution products, and surface active agents. The fourth consists of a miscellaneous group of bases containing disintegrating agents, natural gums, effervescents, glue, fibrin, collagen, etc.

Oily and Fatty Bases.—For many years, cocoa butter (theobroma oil) was used as a suppository base (15). It filled many of the requirements since it was innocuous, bland, nonreactive, and melted at body temperature. Its disadvantages such as rancidity, melting in warm weather, liquefaction when incorporated with drugs, and isomerization to a lower melting point resulted in the constant search for modification or for a better base.

Van Riel and Van der Wielen (16) added wax to cocoa butter in 1913. Then in 1919, Terry (17) suggested the addition of kaolin and a 2% chondrus decoction. In 1919, Behrbalk (18) proposed a substitute for the base of three parts olive oil with one part spermaceti. Rhodehamel (19) suggested coating cocoa butter with gelatin, glycerin, and acacia mixtures to add toughness to the form. A United States patent was obtained in 1924 for a suppository base made by an alcoholic saponification of 3–6% stearic and higher fatty acids dissolved in paraffin oil base (20). "Supositol," a mixture of lard and suet, was suggested as a base by Eshenbrenner (21) in 1927, but this domestic fat quickly became rancid and was not a good substitute (22, 23).

Cooper (24) suggested the addition of 3% calcium oleate to cocoa butter to facilitate the absorption of water into cocoa butter and paraffin suppositories. Hollow forms were introduced by Smith (25) in 1930. The medication was filled into the hollow shell and sealed. Following the lead of Cooper concerning water absorption into the base, Schroff (26) discussed the use of lecithin and cholesterol in conjunction with cocoa butter.

"Astra-fat" (27), a hydrogenated peanut oil melting at 38–41°, was introduced in 1934 as a suppository base. In 1937, a patent was granted in Austria to Kremel (28) for a base composed of fat dispersed in a colloidal gelatin mass. The same year, a Russian (29) gave a formula for calculating the amounts of wax and fats to be mixed to obtain various melting points. These mixtures were to be used as substitutes for cocoa butter.

At this time a U. S. patent was granted Nitardy, Christiansen, and Deuble (30) and assigned to E. R. Squibb & Sons for an oleaginous base comprised of combinations of hydrocarbons with the ammonium salts of sulfonated olein. This agent aided in lowering surface and interfacial tension, allowed emulsification, and lent a water-solubility characteristic.

Ferraris (31) in 1938 suggested the addition of cetyl alcohol and other higher molecular weight alcohols. The following year he adapted cocoa butter bases further by addition of cod-liver oil, triethanolamine stearate, and wax (32). The same year, Hediger and Lehmann (33) gave formulas for glycerin-cocoa butter bases.

Jermstad and Fretheim (34) discussed the addition of beeswax and spermaceti to affect the melting point of cocoa butter. The problem of a suppository base for tropical climates brought forth the work by Caldwell (35) suggesting hydrogenated palm-kernel oil and hydrogenated soybean oil, the latter when no oil-soluble drugs are incorporated. Muzel (36) developed a cocoa butter substitute by the purification of palm-kernel oil to obtain its stearins. He claims a stable base with none of the physical incompatibilities exhibited by cocoa butter.

Because of the war in 1941, there was a need for a cocoa butter substitute for blockaded countries. Buchi and Oesch (37) listed some substitutes such as wool fat, paraffin, olive oil, poppy oil, stearyl alcohol, cetyl alcohol, and waxes. At that time Hoffmann-LaRoche and Ciba had patented two bases in Switzerland. One patent was for synthetic fatty acid esters of propylene glycol. The other was a combination of sodium acetate and urea emulsified with lecithin and acacia or tragacanth.

In 1942, Rae (38) reported the addition of 10% Lanette Wax SX (a mixture of cetyl and stearyl alcohols as partial phosphoric acid esters) which allowed the addition of large volumes of water to the theobroma oil base.

Buchi and Oesch (39) in 1943 discussed the *cis-trans* isomerization of melted cocoa butter to produce the elaidic acid isomer from the fatty acid form, resulting in a lowered melting point of 28°.

Waxman and Eiler (40) described the use of Aerosol, Lanette Wax SX, triethanolamine stearate, and glyceryl monostearate as emulsifiers for cocoa butter bases. They stated that glyceryl monostearate is most effective, and that the emulsifiers are used to best advantage in concentrations of 10%.

In an anonymous article (41) from South America in 1946, the use of a froth emulsion of cocoa butter poured into molds was suggested. A Swiss patent (42) was obtained in 1948 for a fatty material, melting at body temperature, consisting of mixtures of various acylamino cyclohexanols or their alkyl esters. In 1949, Schneider (43) outlined the use of neutral glycerol esters of saturated acids melting at 34–36°. A more recent addition to the fatty-type cocoa butter substitutions was a product proposed in 1950 called "Schluter 200," a fat melting at 37° (44).

Some additional substances proposed as suppository bases are coconut stearin, hydrogenated peanut oil, and mixtures of oleic and stearic acids. Two new names, "Lasupol" and "Suppobasin," were introduced to the long list of theobroma oil substitutes. Hofmann and Hornbogen (45) conducted a pharmacological investigation of synthetic suppository bases and gave information as to their constituents. "Lasupol" has the formula Cetyl—OOC—C_6H_4—COO—Cetyl, and melts at body temperature. "Suppobasin" consists of a mixture of "oxywachs." It is a hydrogenated mineral wax (Montan wax) contained to the extent of 10–18% in lignite. Zschimer and Schwartz Produkte—Germany offer it in grades K-10, K-20, and K-50. These bases are water insoluble, but melt at body temperature. Karber (46) examined these substances and claimed they are nontoxic.

Statements by a number of authors concerning cocoa butter as a base indicate that this vehicle is fast diminishing in use. In commercial manufacture of suppositories, theobroma oil has been almost entirely superseded. The use of hydrogenated products without the tendency to rancidify, with the desired melting point, and without the isomeric change to a lower melting point when heated, is replacing this base to a great extent. However, the fatty or oily type base will never be entirely replaced because it offers a useful vehicle for oil-soluble drugs intended for local action or for slow, prolonged absorption.

Glycerin-Gelatin Bases.—Among the glycerin-gelatin type bases there are two distinct classes: (*a*) Glycerin-gelatin and sodium stearate mixtures intended primarily for prompt evacuation; and (*b*) glycerin-gelatin water bases to which medicaments are added. The first class (47) is best demonstrated by the glycerin suppositories U. S. P. available in every drugstore and used as an enematic. Their action can be ascribed to the irritation of the mucous membrane caused by the glycerin-stearate and does not depend on any drug. The second class of bases is coming into wide use, especially for the manufacture of vaginal products.

The glycerin-gelatin water base is often adopted when other bases become too brittle for use because of incorporated drugs. The combination of glycerin and gelatin itself is too firm for use as a suppository base; thus only one-half the weight of the product consists of this base material, the remainder being water. This vehicle lends itself especially well to water-soluble drugs (5) and the incorporation of solid extracts.

Many modifications of these two classes have been suggested to correct hygroscopicity, transparency, brittleness, and temperature resistance to shrinkage and "weeping." Scoville (48) in 1925 suggested the inclusion of 10% water in glycerin-sodium stearate suppositories to give them transparency. Agar and acacia have been suggested as adjuncts to the base to add firmness (49, 50). Several methods have been given for the determination of glycerin in these types of bases. One method is outlined by Kaserman and Blum (51).

With the increased use of glycerin in munitions, a lesser production of glycerin from the saponification process of soap production, and a rise in the use of detergents, there has been much interest in the use of glycerin substitutes for these bases. Propylene glycol (52), sorbitol or mannitol (53), and 2,3-butylene glycol (54) were discussed in this regard.

Water-Soluble or Miscible Bases.—One of the first references to this type of base was found in a German patent issued in 1934 for a suppository excipient composed of cholesterol, lactose, and water heated to 80–90°, then rapidly cooled (55). Of greater importance was the patent granted to Hoffmann-LaRoche in 1936 (56) for work done by Bird on glycerol and glycol esters of stearic, lauric, and palmitic acids. This product "Monolene" (57) is hydrophilic and self-emulsifying in water.

A German patent was issued to Nassan (58) for a new base consisting of neutral soluble salts of condensation products of high molecular weight, albumin degradation products, and higher fatty acids, i. e., the sodium salt of stearyllysalbinic acid. Wankmuller (59) described two bases, Suppanol O and ON, which are hydrophilic and will absorb 10% of water. These bases melt at body temperature but are incompatible with tannins and are strong reducing agents.

Of particular interest concerning this type base are the polyethylene glycol polymers. Bochmuhl, Middendorf, and Starch (60) received patents in Germany and the United States in 1937 for a polyethylene oxide polymer and ricinoleic acid mixture with water which they suggested as a suppository base. Realizing the possibilities of the polymer itself as a base, Middendorf (61) described its use as a nontoxic, water-absorbable suppository base which can be stored at high room temperatures. This base, called "Postonal" in Germany, was described as a cocoa butter substitute by Breinlich (62) and Freudweiler (63) in 1941 and by Middendorf (64) in 1944. By 1946 the use of this new base was widespread in Europe and discussions of its incompatibilities, which seem quite numerous, were published by Lehmann (65) and were reviewed in France (66), where this base is called "Scurol" while in the United States it is named "Carbowax". Some of its apparent incompatibilities are phenol, resorcinol, peru balsam, and tannins.

In an article by Cheymol, Buffet, and Lechat (67), the addition of 10% of water was suggested to facilitate solution of the suppository after insertion. Kunert and Awe (68) made the statement that Postanol is not as chemically inert as it was originally claimed. They identified this product as a polymer of ethylene oxide containing about 100 ethylene oxide molecules.

In 1949, Gillham and Tomlinson (69) described a base consisting of 1 part Carbowax 1500 with 2 parts Carbowax 6000. The following year several articles were published on the toxicity of these polymers. Straus (70) described sensitivity to polyethylene glycols, and Smyth and Carpenter (71) did intracutaneous and eye conjunctival tests on these bases. Reports of many workers on toxicity of these polymers indicated little difference in sensitivity to individual bases, but a diminished reaction with Carbowax 6000. The problem resolves in the use of the "W" grades of these products. These water-white, purified polymers exhibit no primary irritation to the conjunctiva of a rat eye (71). It may be deduced from this information that the sensitivity to these bases is due to impurities and not to the base itself. The "W" grades of Carbowaxes are recommended for use as suppository bases. Manz (72) published an article in 1950 in which he referred to Postonal W, indicating the adoption of this purified form.

Closely related chemically to the Carbowax bases are certain products called "Tweens." They may be described, as in the patent granted Wm. S. Merrell Co. in the name of Shelton and Magid (73), as the reaction products of polyhydric alcohols with sorbitol or mannitol stearate or other fatty-acid esters with at least twelve carbon atoms. Eaton Laboratories were given a patent for an adaptation of this original base (74). The base they proposed is a mixture of 90 parts sorbitan monostearate hydroxy polyethylene ether with 10 parts of either sorbitan monoricinoleate, mineral oil, or glyceryl laurate. Ward (75) discussed the use of Tweens as a hydrophilic suppository base, saying they are nontoxic, compatible, nonirritating, have good storage qualities for at least a year, and form stable oil-in-water emulsions with aqueous fluids. He suggested some formulas for products which melt at 37° in ten to fifteen minutes; 60% Tween 61 with 40% Tween 60, and 90% Tween 61 with 10% glyceryl laurate.

A recent article by Dolder (76) mentioned the use of Tween 61, Tween 20, and Carbowax 1500 W. He predicts that synthetic bases will soon replace natural products. A similar discussion of the uses of Tweens and Carbowax occurred in a very recent publication (77). These bases are substantially unaffected by temperature but derive their action by abstraction of water from adjacent tissue and release of the medicament from the vehicle in the presence of the abstracted moisture.

The effort of Saunders and Raymond (78) to provide a suppository base that would quickly melt or dissolve, yet have good storage quality at high temperatures, resulted in a patent granted for mixtures of polymers of polyethylene glycol of high molecular weight with hydrogenated oils, glycol esters, eicosane, cocoa butter, lanolin; or fatty acid esters. The mixture of two insoluble solids with high and low melting points was thought by these investigators to provide good storage quality with ready ability to melt when inserted.

Another type of water-soluble suppository vehicle was suggested by Davies (79), who described the use of water-soluble cellulose derivatives in the form of methylcelluloses and sodium carboxymethylcellulose.

Water-soluble or water-dispersible bases seem to be the answer to a "universal suppository base." They offer a vehicle for water-soluble and oil-soluble medication in a stable, nonreactive, nontoxic base in a physical state that will allow storage and handling at elevated temperatures.

Miscellaneous Bases and Coatings.—A variety of bases not falling into the three classes previously discussed are presented here. One of the first of these innovations was discussed in a patent awarded Fitzgerald (80) for an effervescent suppository base which contained citric acid and sodium bicarbonate. The ingredients were mixed dry and compressed into the desired form. This idea was further adapted in a British patent granted Elsworth and Luber (81) for a suppository containing an inner pellet of dry tartaric acid and sodium bicarbonate dispersed in cocoa butter surrounded by a carragenin-gelatin and medicinal shell. When introduced, the absorption of water ruptures the suppository, producing a froth containing the medication. This form was particularly useful as a contraceptive.

Lannung (82) gave a formula for making elastic suppositories composed of white gelatin, kaolin, glycerin, and water. Koninklyije (83) suggested the use of fibrous materials which swell in the presence of moisture such as collagen impregnated with the medicinal. An interesting patent was granted in Britain for an insulin suppository containing lactic acid as a buffer to protect the insulin from destruction in the media of the rectum (84).

Schmidt (85) was granted a U. S. patent on a mixture of urea, lactose, agar, and tragacanth molded to produce preformed suppositories which could be used as containers for medication.

In a discussion (86) of the substitution of glue for gelatin in suppositories, the statement was made that the two products are similar and differ only in gradation, glue being gelatin plus chondrin and mucin. Methods for the sterilization of glue and the preparation of glycero-glue suppositories were given.

Middendorf (64) described the use of polymerized vinyl alcohol precipitation products as substitutes for agar and as excellent suppository and pill coatings. Fessendon (87) used this substance as a suppository coating in 20% concentrations in conjunction with 1% of a nonionic surface active agent, such as a Span.

MANUFACTURING PROCEDURES

Since J. U. Lloyd devised a suppository mold in 1865, there have been many methods suggested by which these dosage forms may be produced (88). Prior to that time, molded suppositories were prepared by pouring the molten mass into paper cones. Several machines have been suggested to prepare these medicinal forms mechanically (89, 90); however, many pharmaceutical houses in the United States use molds which will produce 100 suppositories per pouring. It is largely a hand operation. Details for large-scale manufacture of suppositories are available in pharmaceutical texts such as Husa (12).

Research by Jermstad and Fretheim (91) indicated that suppositories prepared by molding had less discrepancy in dosage than those made by any other method. The molded form has the best appearance (92) and is easiest to prepare. However, care must be taken in the manner in which the mold is filled, since the method of filling affects the capacity of the mold (93).

A lubricant may be used if the preparation has a tendency to stick to the surfaces of the mold. A proposed formula for such a lubricant was given by Wooley and Forrester (94). It contains castor oil, 1 part; hard soap, 2 parts; alcohol, 18 parts; and water, 2 parts. A simple alcoholic soap solution may also be used, but the use of clean, dry, chilled molds usually eliminates the need for a lubricant. The newer types of bases, such as Carbowaxes and Tweens, shrink slightly on cooling, thus drawing away from the mold and obviating the use of a lubricant.

To insure the accuracy of the amounts of medicament in the dosage form, much study has been done on the specific gravity and the relative displacement of the base by medicaments (95–98).

Goldman and Guidini (99) discussed some difficulties in incorporating dried extracts into suppositories. They suggested that these extracts be dissolved in or emulsified with a portion of the melted base prior to incorporation.

Because of the chemical and physical nature of suppository bases, microbial spoilage often occurs. Smith (100) gave some tests for purity of gelatin in suppositories. Eshenbrenner (101) discussed the addition of *p*-hydroxy benzoate to prevent fungus growth; while Buchi (102) suggested the use of "Nigapen," methyl paraben. Several British workers stated that the incorporation of aluminum stearate into oils and fatty suppository bases produced a bacteriostatic effect (103).

PHYSICAL AND BIOLOGICAL TESTS

Some work has been done to standardize and assay suppository bases as to liberation of ingredients, therapeutic activity, melting point, and disintegration. Baker and Ransom (104) gave a test for the disintegration rate of this medicinal form. Recently Malangeau (105) gave a method for the determination of the melting point of suppositories. A wire of small diameter is placed into the mold before the form solidifies. The form is immersed in water and held by the wire, and the temperature of the liquid is raised slowly (1° every two to three minutes) until the suppository slips off the wire. The temperature at this point is the melting point. He also presented a method to determine the mechanical strength of the mass. A cylinder 9 x 15 mm. is cut from the suppository and subjected to compression. This test determines the collapsing load at a constant temperature.

An early method of testing the physiological absorption of medicaments from the suppository base was discussed by Rapp (106). He listed various suppository masses, and described a test utilizing 50 mg. of methylene blue absorbed from the dosage form and recognized by discoloration of the urine. This test was reiterated in a more recent article (67).

Buchi and Schlumpf (107) gave details for a microbiological test for absorption of the medicinal This test utilizes an antiseptic which develops a zone of inhibition on an agar plate inoculated with a microorganism. Ward (75) discussed a similar test using the U. S. P. cup method producing a zone of inhibition caused by release of the drug from the tested suppository.

A simple test for primary irritation of mucous membrane was given recently (71), involving the introduction of the test vehicle into the eye of a rat. The conjunctiva is checked for signs of irritation. A more involved method for testing the release of medicament from the base was suggested by Charonnat, Chevillard, and Giono (108), who introduced methyl nicotinate into guinea pigs in the form of a suppository and measured the rise in temperature of this test animal by a thermocouple attached to its ear.

CONCLUSIONS

Although there may always be a need for the fat-type suppository base, especially for local acting and oil-soluble drugs, research should be directed to a greater extent to the newer types of bases, such as the water-soluble resins and other polymerized compounds, some of the silicones, various carbohydrates, and proteins and solid emulsions.

The old idea that a suppository base should melt at body temperature may become obsolete, for such bases are not necessary for therapeutic action nor are they stable in warm climates.

REFERENCES

(1) Webster, N., "Webster's Collegiate Dictionary," 5th ed., G. & C. Merriam Co., Springfield, Mass., 1941, p. 1002.
(2) Kremers, E., and Urdang, G., "History of Pharmacy," J. B. Lippincott Co., Philadelphia, 1947, p. 7.
(3) Sollman, T., "Manual of Pharmacology," 5th ed., W. B. Saunders Co., Philadelphia, 1937, p. 37.
(4) Davison, F. R., "Handbook of Materia Medica, Toxicology, and Pharmacology," 4th ed., C. V. Mosby Co., St. Louis, 1949, p. 19.
(5) Caspari, C., and Kelly, E., "Treatise on Pharmacology," 8th ed., W. B. Saunders Co., Philadelphia, 1939, p. 475.
(6) Anon., *Bull. gen. de Therapeut.*, **38,** 179(1928).
(7) Robinson, G. D., *J. Pharmacol. Exptl. Therap.*, **32,** 81(1927).
(8) Macht, D. I., *ibid.*, **10,** 509(1918).
(9) Lesser, M. A., *Drug & Cosmetic Ind.*, **53,** 32(1943).
(10) Park, J. H., *J. Pediat.*, **23,** 326(1943).
(11) Lesser, M. A., *Drug & Cosmetic Ind.*, **54,** 403(1944).
(12) Husa, W. J., "Pharmaceutical Dispensing," 3rd ed., Husa Bros., Iowa City, 1947, p. 156.
(13) Buchi, J., and Oesch, P., *Pharm. Acta Helv.*, **20,** 129(1945).
(14) McCormick, E. J., and Ramsey, T. L., *J. Am. Med. Assoc.*, **116,** 817(1941).
(15) Salomon, B., *Pharm. Ztg.*, **58,** 502(1913).
(16) Van Riel, J., and Van der Wielen, P., *Pharm. Weekblad*, **1912,** 25.
(17) Terry, H., *Pharm. Ztg.*, **63,** 256(1918).
(18) Behrbalk, L., *Chem. Zentr.*, **II,** 760(1918).
(19) Rhodehamel, H. W., U. S. pat. 1,366,941 (1921).
(20) Crane, F. D., and Schieffelin, W. J., U. S. pat. 1,499,348 (1924).
(21) Eshenbrenner, H., *Pharm. Ztg.*, **70,** 1791(1925).
(22) Muller, H. A., *Deut. Apoth. Ztg.*, **53,** 728(1938).
(23) Lehmann, H., *Schweiz. Apoth. Ztg.*, **81,** 201(1943).
(24) Cooper, J., *Pharm. J.*, **117,** 371(1926).
(25) Smith, I. J., *ibid.*, **122,** 632(1930).
(26) Schroff, E., *Pharm. Ztg.*, **76,** 1239(1931).
(27) Gfeller, H., *Pharm. Acta Helv.*, **9,** 13(1934).
(28) Kremel, A., Austrian pat. 149,187 (1937).
(29) Anon., *Mfg. Chemist*, **8,** 349(1937).
(30) Nitardy, F. W., Christiansen, W. G., and Deuble, J. L., U. S. pat. 1,995,776 (1935).
(31) Ferraris, A., *Boll. chim. farm.*, **77,** 725(1938).
(32) Ferraris, A., *ibid.*, **78,** 379(1939).
(33) Lehmann, H., and Hediger, F., *Pharm. Acta Helv.*, **14,** 83(1939).
(34) Jermstad, A., and Fretheim, B., *Quart. J. Pharm. Pharmacol.*, **13,** 186(1940).
(35) Caldwell, A. F., *ibid.*, **12,** 680(1939).
(36) Muzel, K., *Schweiz. Apoth. Ztg.*, **90,** 125(1952).
(37) Buchi, J., and Oesch, P., *ibid.*, **79,** 385(1941).
(38) Rae, J., *Pharm. J.*, **148,** 13(1942).
(39) Buchi, J., and Oesch, P., *Pharm. Acta Helv.*, **18,** 333(1943).
(40) Waxman, P., and Eiler, J. J., *J. Am. Pharm. Assoc., Pract. Pharm. Ed.*, **6,** 232(1945).
(41) Anon., *Gaz. farm. (Rio)*, **15,** 22(1946).
(42) Basle University, Swiss pat. 215,239 (1948).
(43) Schneider, E., *Süddeut. Apoth. Ztg.*, **88,** 431(1948).
(44) Wankmuller, A., *Pharm. Ztg.*, **86,** 343(1950).
(45) Hofmann, H., and Hornbogen, U. H., *Pharm. Zentralhalle*, **89,** 369(1950).
(46) Karber, G., *Arch. exptl. Path. Pharmakol.*, **212,** 509(1951).
(47) Prout, W. A., THIS JOURNAL, **25,** 1123(1936).
(48) Scoville, W. L., *ibid.*, **13,** 818(1924).
(49) Pegurier, G., and Luisi, M., *Bull. pharm. Sud-Est.*, **44,** 28(1940).
(50) Ulrich, A., *Chemist and Druggist*, **101,** 913(1924).
(51) Blum, C., and Kasermann, H., *Pharm. Acta Helv.*, **21,** 233(1946).
(52) Tice, L. F., and Moore, A. W., J. AM. PHARM. ASSOC., *Pract. Pharm. Ed.*, **11,** 565(1950).
(53) Muralti, G. A., *Boll. chim. farm.*, **89,** 169(1950).
(54) Zwikker, J. J. L., *Pharm. Weekblad*, **78,** 1261(1941).
(55) Weil, O., German pat. 583,337 (1934).
(56) Bird, J. C., U. S. pat. 2,055,063 (1936).
(57) Bird, J. C., THIS JOURNAL, **26,** 475(1937).
(58) Nassan, M., German pat. 674,980 (1939).
(59) Wankmuller, A., *Süddeut. Apoth. Ztg.*, **89,** 373(1949).
(60) Bockmuhl, M., Middendorf, L., and Starch, W., German pat. 650,000 (1937); U. S. pat. 2,149,005 (1937).
(61) Middendorf, L., *Munch. med. Wochschr.*, **86,** 95(1939); through *Chem. Abstr.*, **45,** 4742(1939).
(62) Breinlich, J., *Deut. Apoth. Ztg.*, **55,** 397(1940).
(63) Freudweiler, R., *Schweiz. Apoth. Ztg.*, **79,** 149(1941).
(64) Middendorf, L., *Chem. Zentr.*, **II,** 440(1943).
(65) Lehmann, H., *Pharm. Acta Helv.*, **21,** 355(1946).
(66) Anon., *Ann. pharm. franç.*, **1,** 55(1943).
(67) Cheymol, J., Buffet, J., and Lechat, P., *ibid.*, **5,** 59(1947).
(68) Kunert, G., and Awe, W., *Pharm. Ztg.*, **85,** 322(1949).
(69) Gillham, R. W., and Tomlinson, J. E., *Pharm. J.*, **162,** 472(1949).
(70) Straus, M. J., *Arch. Dermatol. Syphilol.*, **61,** 420(1950).
(71) Smyth, H. F., Carpenter, C. F., and Weil, C. S., THIS JOURNAL, **39,** 349(1950).
(72) Manz, E., *Süddeut. Apoth. Ztg.*, **90,** 321(1950).
(73) Shelton, R. S., and Magid, L., U. S. pat. 2,241,331 (1941).
(74) Ward, W. C., and Scott, A. B., U. S. pat. 2,469,618 (1949).
(75) Ward, W. C., THIS JOURNAL, **39,** 265(1950).
(76) Dolder, R., *Schweiz. Apoth. Ztg.*, **89,** 381(1951).
(77) Anon., *Am. Profess. Pharmacist*, **18,** 140(1952).
(78) Saunders, F. J., and Raymond, A. L., U. S. pat. 2,538,127 (1951).
(79) Davies, R. E. M., *Pharm. J.*, **165,** 347(1950).
(80) Fitzgerald, G., U. S. pat. 1,878,766 (1933).
(81) Elsworth, F. C., and Luber, L. J., British pat. 459,327 (1937).
(82) Lannung, A., *Arch. Pharm. Chemi*, **45,** 613(1938).
(83) Koninklyije, N. V., British pat. 487,258 (1939).
(84) Van Aalst, D. J., British pat. 499,824 (1936).
(85) Schmidt, K. W., U. S. pat. 2,186,729 (1940).
(86) Anon., *Pharm. J.*, **149,** 41(1942).
(87) Fessendon, G. R., U. S. pat. 2,477,292 (1949).
(88) Lloyd, J. U., THIS JOURNAL, **9,** 595(1920).
(89) Thieme, A., *Apoth. Ztg.*, **45,** 37(1920).
(90) Gartner and Schenker, *Schweiz. Apoth. Ztg.*, **73,** 357(1935).
(91) Jermstad, A., and Fretheim, B., *Arch. Pharm. Chemi*, **47,** 239(1940).
(92) Buchi, J., and Oesch, P., *Pharm. Acta Helv.*, **20,** 497(1945).
(93) Andrews, M. J., THIS JOURNAL, **23,** 1003(1934).
(94) Wooley, S. W., and Forrester, G. P., "Pharmaceutical Formulations," Vol. I, 10th ed., London, 1929, p. 537.
(95) Rae, J., *Pharm. J.*, **121,** 315(1928).
(96) Roberts, I., *Chemist and Druggist*, **116,** 300(1932).
(97) Roberts, R. A., and Ostins, W. T., *Pharm. J.*, **148,** 112(1942).
(98) Buchi, J., *Pharm. Acta Helv.*, **20,** 403(1945).
(99) Goldman, J., and Guidini, C. A., *Schweiz. Apoth. Ztg.*, **74,** 709(1936).
(100) Smith, P. I., *Pharm. J.*, **122,** 617(1929).
(101) Eshenbrenner, H., *Pharm. Ztg.*, **72,** 467(1927).
(102) Buchi, J., *Schweiz. Apoth. Ztg.*, **82,** 469(1944).
(103) Couthard, C. E., Chantrell, B. H., and Crashaw, B., *J. Pharm. Pharmacol.*, **3,** 215(1951).
(104) Baker, J. R., and Ranson, R. N., *J. Hyg.*, **34,** 474(1934).
(105) Malangeau, P., *Ann. pharm. franç.*, **6,** 50(1948).
(106) Rapp, V., *Pharm. Ztg.*, **72,** 312(1927).
(107) Buchi, J., and Schlumpf, R., *Pharm. Acta Helv.*, **19,** 171(1944).
(108) Charonnat, R., Chevillard, L., and Giono, H., *Ann. pharm. franç.*, **7,** 627(1949).

A Study of Suppository Bases. II. A Colorimetric Method for Measuring Medicinal Release from Suppository Bases*,†

By HERBERT MICHEAL GROSS and CHARLES H. BECKER‡

Suppositories of previously reported bases were made by the fusion method, and their physical appearance, stability, and rate of medicinal release were compared. Several new bases were developed and tested; some polyoxyethylene stearate analogs and some monostearate esters of polyethylene glycol polymers were found to have excellent properties. The colorimetric method developed and used in this investigation serves as one standard for determining the relative value of a suppository base in regard to the release of its active medicament, but is limited to an *in vitro* study.

FOLLOWING a review of the literature on suppository bases (1), a number of well-known bases and several new ones were prepared and evaluated colorimetrically for release of active constituents.

To determine the quickness of release of medicament from the base, suppositories of various bases and formulas were prepared including a known amount of water-soluble dye which simulated a water-soluble medicament, allowing a comparative basis for the estimation of medicinal release from various bases.

EXPERIMENTAL

Preparation of Suppositories.—All suppositories were prepared by the fusion process. When the melted base showed a tendency to solidify, they were poured into chilled molds. A suppository with an accurately weighed amount of dye and a blank were prepared from each base. Suppositories in U. S. P. XIV were prepared according to official methods. The same mold lubricant (2) was used throughout the investigation and the included dye was from the same lot number and sample in all cases.

Suppositories were stored at least two weeks at refrigerator temperature before testing to allow for the isomerization of such bases as theobroma oil.

Materials Used.—Wherever possible, official chemicals were used in this investigation. Nonofficial products employed were food and drug grade. The citrus pulp used was prepared in our laboratory.

Testing Technique.—Before testing, the suppositories were removed from the refrigerator and allowed to come to room temperature. The suppository containing the dye and its corresponding blank were placed in duplicate 500-ml. glass, Oldberg-type percolators stoppered at orifice, containing a measured quantity of distilled water, and mounted in a constant temperature bath set at 36.4 to 37.4°. The containers were equipped with laboratory stirrers operating at the same low speed. This apparatus provided for the removal of samples and maintained uniform concentration of liquid. As the suppository melted or dissolved, samples were taken at varied time intervals.

The samples, withdrawn by pipette, were drained uniformly into volumetric flasks and brought to volume. Each solution was placed in a colorimeter tube previously rinsed twice with the particular dilution. Readings of these tubes were made on a Klett-Summerson Photo-Electric colorimeter with No. 54 green filter, with allowance made for the blank.

The use of a blank corrected for any error that may have been introduced by solution of the base, the distilled water used, or the mold lubricant causing absorption of some of the transmitted light beam.

Since the *p*H of the rectal contents varies with the type of food ingested, the extent of decomposition of digestive by-products, and the degree of dehydration of fecal material, it was impossible to set a *p*H at which rectal absorption might be simulated. Therefore, distilled water of the same source and *p*H was used throughout.

The test apparatus was designed only to allow a comparative study under controlled conditions and limit the variable factors of this investigation, and was not intended to act as an artificial rectum.

Calculation of Data.—So that results could be expressed in mg./ml., a graph of known concentrations of dye vs. colorimeter readings was prepared. An absorption line was plotted with amaranth, F. D. & C. Red No. 2.

The range within which this dye obeys the Beer-Lambert law was used as the limiting factor. From the graph prepared, the concentration of dye released from the bases was expressed in mg./ml.

Taking into consideration the size sample withdrawn, the amount of dye in the withdrawals, and the dilution, the milligrams of dye released during each time interval was calculated. The ratio of dye released to dye present in the suppository indicated the percentage release. Data were expressed as percentages to allow for variable factors among experimentors.

Results of tests performed on the suppository bases and formulas tested are given in Tables I-IX.

The appearance of the molded product was noted and listed in the tables. Where no comment appears, the product was considered acceptable. The heading "Reference" refers to bases reported in the

* Received July 31, 1952, from the College of Pharmacy, University of Florida, Gainesville.

† Abstracted in part from a dissertation to be presented to the Graduate Council of the University of Florida by Herbert Micheal Gross in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

‡ Associate Professor of Pharmacy, College of Pharmacy, University of Florida.

TABLE I.—THEOBROMA OIL-TYPE SUPPOSITORY BASES

Base	Reference	Melting Range, °C.	Appearance	Per Cent Dye Released with Time in Min. 1	2	3	4	6	8	10
Theobroma oil, U. S. P.	..	30–35	....	10.7	22.1	36.4	..	39.0	42.3	45.8
Theobroma oil with 5% white wax, U. S. P.	(3)	35–40	....	8.1	12.4	..	30.1	35.4	38.2	39.0
Theobroma oil with 2.5% cetyl alcohol	(4)	29–32	....	0	10.9	27.7	34.4	36.2	41.5	43.1
Theobroma oil with 1% Aerosol OT[a]	(5)	30–35	....	41.3	65.4	65.4	68.8	71.6	71.6	71.6
Theobroma oil with 10% water	New	30–32	....	2.8	21.1	39.4	52.2	57.9	61.3	61.9
Theobroma oil with 7% water and 3% calcium oleate	(6)	29–32	....	12.0	21.3	..	38.4	..	62.2	62.6
Theobroma oil with 2% water and 1% lecithin	(7)	28–30	Soft	12.8	65.4	68.0	70.0	70.0	70.0	70.0
Theobroma oil with 2% water and 2% cholesterol	(7)	34–37	Speckled	2.8	6.0	7.5	..	10.7	10.7	10.7
Theobroma oil with 10% citrus pulp	New	31–35	Grainy	8.3	49.0	72.3	82.5	92.5	92.5	92.5

[a] American Cyanamid Co.

TABLE II.—HYDROGENATED OILS AS SUPPOSITORY BASES

Base	Reference	Melting Range, °C.	Appearance	Per Cent Dye Released with Time in Min. 1	2	3	4	5	8	11
Hydrogenated soybean oil[a]	..	34–36	Unmoldable	..	..	..	..	..	..	..
Hydrogenated coconut oil[b]	..	37–40	.....	11.5	58.8	60.8	62.0	62.0	62.0	62.0
Hydrogenated cottonseed oil[a]	..	36–40	.....	2.1	2.1	5.2	..	6.1	13.5	25.3
Hydrogenated coconut oil[a]	..	36–39	.....	5.3	5.3	7.3	..	11.0	11.0	13.6
Hydrogenated coconut oil ester[b]	..	37–40	.....	0	2.6	4.8	14.1	14.9	..	17.1

[a] Vegetable Oil Products Co., Inc.
[b] E. F. Drew & Co., Inc.

TABLE III.—GLYCERIN-STEARATE AND GLYCERO-GELATIN AS SUPPOSITORY BASES

Base	Reference	Melting Range, °C.	Appearance	Per Cent Dye Released with Time in Min. 1	2	4	6	9	11
Glycerin stearate suppositories, U. S. P.	..	..	..	7.2	10.2	19.2	22.9	29.1	32.4
Glycerin stearate base with 10% water	(8)	..	..	7.5	10.6	16.2	26.7	29.5	35.3
Glycerinated gelatin suppositories, U. S. P.	..	..	..	9.1	20.9	45.0	83.3	86.0	86.0

literature. Bases not previously reported in the literature are listed as "new." Melting ranges were taken from manufacturers' literature where possible, or were determined by U. S. P. XIV methods. Manufacturers of specialty products have been identified in each case.

RESULTS

Table I.—Results indicate that the addition of cholesterol, cetyl alcohol, and wax decreased the percentage release of medicament from theobroma oil bases. The addition of a surface active agent such as Aerosol OT and the inclusion of a suppository disintegrator (14), of the same nature as a tablet disintegrator, resulted in marked increases. The simple physical admixture of water with theobroma oil gave results comparable to those obtained by the use of calcium oleate and lecithin emulsifiers.

Table II.—Data indicate that hydrogenated oil bases are not suitable substitutes for theobroma oil because of their low percentage of medicinal release.

Table III.—A study of the official U. S. P. glycerin suppositories was included. The glycerinated gelatin base gave a high and complete percentage of release, but is incompatible in a great many cases.

TABLE IV.—CARBOWAX[a] TYPE SUPPOSITORY BASES

Base	Reference	Melting Range, °C.	Appearance	Per Cent Dye Released with Time in Min. 1	2	3	5	7	10
Carbowax 4000 W	(9)	50–55	Fissured	1.2	6.2	9.8	20.6	33.8	49.8
Carbowax 4000 W with 10% water	(10)	47–50	....	4.4	12.6	20.4	38.2	55.3	67.0
Carbowax 4000 W with 10% citrus pulp	New	51–55	Grainy	4.4	18.8	22.6	42.2	65.8	77.0
Carbowax 4000 W with 50% Carbowax 1500 W	(11)	43–47	....	4.4	7.9	13.2	20.4	31.1	45.2
Carbowax 6000 W	(9)	58–62	Fissured	2.4	5.0	7.5	21.1	36.2	49.7
Carbowax 6000 W with 50% Carbowax 1500 W	(11)	47–49	....	7.5	22.6	38.7	57.8	68.8	77.2
Carbowax 6000 W with 10% water	(10)	56–59	Fissured	6.3	10.1	21.7	35.9	50.3	61.3

[a] Carbide & Carbon Chemicals Corp.

TABLE V.—GLYCEROL AND GLYCOL ESTERS[a] AS SUPPOSITORY BASES

Base	Reference	Melting Range, °C.	Appearance	Per Cent Dye Released with Time in Min. 1	2	4	6	8	10
Propylene glycol monostearate	(12)	43–46	Wrinkled	1.0	1.2	3.9	9.0	18.0	18.0
Propylene glycol monostearate with 10% water	New	40–42	Wrinkled	..	9.0	17.2	21.6	26.8	34.8
Glyceryl monostearate	(12)	51–61	Wrinkled	0	0	0	0.2	5.9	5.9
Glyceryl monostearate with 10% water	New	55–60	Wrinkled	1.0	1.0	1.3	1.3	1.3	1.9
Glyceryl monostearate S	(12)	57–61	Wrinkled	0.9	1.1	1.9	9.4	13.3	18.0
Glyceryl monostearate S with 10% water	New	55–58	....	1.4	1.9	11.4	18.4	23.0	23.6
Diethylene glycol stearate	New	54–59	Wrinkled	2.2	4.4	5.3	8.9	15.1	19.5
Diethylene glycol stearate with 10% water	New	52–55	Wrinkled	3.9	4.1	6.2	6.2	7.1	7.2

[a] Glyco Products Co., Inc.

TABLE VI.—POLYETHYLENE GLYCOL ESTERS AS SUPPOSITORY BASES

Base	Reference	Melting Range, °C.	Appearance	Per Cent Dye Released with Time in Min. 1	2	4	6	8	10
Carbowax 1000 monostearate[a]	New	39–42	....	0.9	2.3	6.7	32.6	53.8	62.3
Carbowax 1000 monostearate[a] with 10% water	New	38–40	....	0.9	4.4	23.1	31.7	32.4	32.4
Carbowax 4000 monostearate[a]	New	52–54	Fissured	0	2.4	5.6	11.9	17.8	20.5
Carbowax 4000 monostearate[a] with 10% water	New	47–49	....	5.9	10.0	20.2	32.4	35.7	54.4
Polyethylene glycol 1540[b] monostearate	New	41–44	....	1.7	5.5	18.7	40.2	44.2	53.3
Polyethylene glycol 1540[b] monostearate with 10% water	New	38–40	....	1.4	1.6	5.4	9.5	13.6	14.6
Polyethylene glycol 1540[b] monostearate with 10% water and 10% polyethylene glycol 300 dioleate	New	39–41	....	0.5	1.0	4.4	15.0	15.0	15.0
Polyethylene glycol 1540[b] monostearate with 10% polyethylene glycol 300 dioleate	New	40–42	....	2.5	4.1	18.7	34.0	47.0	53.4
Polyethylene glycol 1540[b] distearate	New	41–44	Fissured	1.3	4.2	6.3	9.4	13.9	21.6
Polyethylene glycol 1540[b] distearate with 10% water	New	39–41	....	6.2	9.5	13.3	16.1	18.2	19.6

[a] Glyco Products Co., Inc.
[b] Kessler Chemical Co., Inc.

The suggested inclusion of 10% water to the glycerin-stearate bases aided only physical appearance, but not medicinal release.

Table IV.—Several accepted Carbowax bases and formulas were tested. The inclusion of 10% water raised the percentage medicinal release and decreased brittleness. The addition of a suppository disintegrator gave good results. The use of the "W" grade of Carbowax is suggested for use in suppositories (15).

TABLE VII.—SPANS[a] AS SUPPOSITORY BASES

Base	Reference	Melting Range, °C.	Appearance	Per Cent Dye Released with Time in Min. 1	3	5	7	9	12
Span 40	New	42–45	Wrinkled	0	0	1.8	3.5	4.2	4.2
Span 40 with 10% water	New	39–41	....	2.3	4.9	5.4	11.3	16.6	23.2
Span 40 with 10% Span 20	New	40–42	....	0	3.1	5.8	5.8	5.8	7.1
Span 40 with 10% water and 10% Span 20	New	38–40	....	2.8	4.6	7.6	22.2	35.4	46.3
Span 60	New	49–53	Wrinkled	2.3	4.1	4.5	4.5	6.8	8.3
Span 60 with 10% water	New	46–49	Wrinkled	0	0	5.2	5.2	6.0	8.3
Span 60 with 10% water and 10% Span 20	New	45–48	Wrinkled	2.3	4.6	8.0	20.0	31.8	45.2
Span 65	New	44–48	Wrinkled	0	0	1.8	1.8	1.8	1.8
Span 65 with 10% water	New	43–45	Wrinkled	0	0.5	0.5	0.5	0.5	0.5
Span 65 with 10% water and 10% Span 20	New	42–44	Wrinkled	0	1.8	2.6	3.0	3.0	3.0

[a] Atlas Powder Co.

TABLE VIII.—TWEEN[a] TYPE SUPPOSITORY BASES

Base	Reference	Melting Range, °C.	Appearance	Per Cent Dye Released with Time in Min. 1	2	4	7	9	11
Tween 61	(12)	35–39	....	1.3	1.3	2.4	5.1	7.0	8.9
Tween 61 with 10% water	New	34–38	Wrinkled	1.0	2.3	3.9	4.6	5.2	6.5
Tween 61 with 20% water	New	30–32	Wrinkled	2.3	3.0	6.5	12.5	18.9	26.2
Tween 61 with Tween 60	(13)	35–38	....	0	1.6	2.2	2.2	2.2	3.7
Tween 61 with 10% Tween 60	New	33–36	....	2.3	2.3	2.3	4.2	4.2	5.2
Tween 61 with 10% glyceryl laurate	(13)	32–34	....	0	1.0	2.8	3.8	4.5	5.3
Tween 61 with 10% glyceryl laurate and 10% water	New	32–44	....	4.5	8.8	31.5	49.2	56.3	59.8

[a] Atlas Powder Co.

TABLE IX.—MYRJS[a] AS SUPPOSITORY BASES

Base	Reference	Melting Range, °C.	Appearance	Per Cent Dye Released with Time in Min. 1	2	3	5	7	9
Polyoxyethylene stearate, G-2151	New	42–45	....	4.1	8.1	19.4	26.8	52.8	55.3
G-2151 with 10% water	New	40–42	....	8.6	60.4	82.0	97.2	97.2	97.2
Polyoxyethylene stearate, G-2152	New	43–45	Fissured	0.8	0.8	5.5	11.9	20.1	25.7
G-2152 with 10% water	New	41–44	Fissured	6.9	8.9	14.2	28.9	36.3	50.5
Polyoxyethylene stearate, G-2153	New	37–42	....	0	2.4	5.1	14.2	22.6	32.4
G-2153 with 10% water	New	35–39	....	1.1	2.0	4.0	19.3	37.8	45.2
Polyoxyethylene sorbital hexastearate, G-1050	New	43–47	Fissured	0	0	0	0	0	0
G-1050 with 10% water	New	43–47	Fissured	0	0	0	0	0	0

[a] Atlas Powder Co.

Table V.—These bases were all inferior to theobroma oil because of their high melting points and limited solubility in water. Addition of 10% water to propylene glycol monostearate gave best results.

Table VI.—The polyethylene glycol esters gave more favorable results than did their lower molecular weight homologs. The Carbowax esters in particular have excellent possibilities because of their high percentage medicinal release and desirable melting points.

Table VII.—In general the Spans appeared to be poor bases. They gave low percentage medicinal release, and in many instances formed products commercially unsuitable. The percentage medicinal release decreases with an increase in molecular weight of the Span. The best results of those bases tested were obtained from a mixture of 80% Span 40, 10% Span 20, and 10% water.

Table VIII.—The use of Tweens as bases appeared to be of no particular advantage. Formulas tested showed a low rate of medicinal release. Fair results were obtained from a mixture of 80% Tween 61, 10% glyceryl laurate, and 10% water.

Table IX.—One of the best bases tested was found in this group of polyoxyethylene stearates. The combination of Myrj with water yielded products of good form and appearance and resulted in almost complete medicinal release in five minutes under experimental conditions. Both Myrj 51 and its analog 52 gave excellent results and have the desirable high melting point range.

DISCUSSION

The therapeutic aim in administering a suppository is to introduce a medicament which must

then be released and absorbed. The manufacturer seeks a marketable product that will withstand shipping, storage, and shelf aging. To reconcile these aims, research has been directed toward a suppository base which is economical and compatible, has a high melting point, possesses good storage qualities, and has the ability to release its medicament.

Although many attempts have been made to improve theobroma oil and to develop new bases, little has been done to evaluate the relative merits of bases. The procedure herein outlined serves as a basis for such evaluation.

The aim of this study has been to evaluate as many of the known bases as possible, and to investigate previously untested substances for better bases. An attempt has been made to find a suppository base with a melting point range suitable for storage and shipping and a high percentage of medicinal release.

In the manipulation of the test apparatus, it must be noted that the rate of stirring in the test receptacles has a marked influence on results. The apparatus was designed so that the duplicate stirrers were controlled to operate at the same speed, and so that each successive test was performed at the same rate as the previous one. By experimental testing, the rate of stirring was established that would maintain a uniform concentration of dye in the test apparatus, but would not appreciably hasten the solution of the tested base.

The results of tests performed on the various bases and formulas were limited to ten- or eleven-minute maximum test times for two reasons; first, it was found that after this time interval the percentage medicinal release usually became almost constant; and second, by the end of that time, the base must have dissolved or melted to eliminate the defecation reflex.

Results of the investigation indicate that the inclusion of a disintegrating agent (14), similar to those used in tablets, can increase the percentage of medicinal release of a high-melting base only fairly water-soluble.

The addition of small percentages of Aerosol OT aided in solution or dispersion and resulted in a higher percentage of medicinal release (5).

Of the previously undeveloped bases, the Carbowax esters and the polyoxyethylene stearate analogs have been found to possess the required properties and will be reported in a later publication.

SUMMARY

1. A colorimetric *in vitro* method for the potential rate of release of medicinal from a suppository base has been developed and studied.

2. Sixty-seven bases, both new and previously reported, have been evaluated by this method.

3. Several new bases, possessing a good percentage of medicinal release and a desirable, high melting point, have been found.

REFERENCES

(1) Gross, H. M., and Becker, C. H., THIS JOURNAL, **42,** 90 (1953).
(2) Wooley, S. W., and Forrester, G. P., "Pharmaceutical Formulations," Vol. I, 10th ed., London, 1929, p. 537.
(3) Van Riel, J., and Van der Wielen, P., *Pharm. Weekblad*, **1912,** 25.
(4) Rae, J., *Pharm. J.*, **148,** 13(1942).
(5) Waxman, P., and Eiler, J. J., J. AM. PHARM. ASSOC., *Pract. Pharm. Ed.*, **6,** 232(1945).
(6) Cooper, J., *Pharm. J.*, **117,** 371(1926).
(7) Schroff, E., *Pharm. Zig.*, **76,** 1238(1931).
(8) Scoville, W. L., THIS JOURNAL, **13,** 818(1924).
(9) Bockmuhl, M., Middendorf, L., and Starch, W., German pat. 650,000 (1937); U. S. pat., 2,149,005 (1937).
(10) Cheymol, J., Buffet, J., and Lechat, P., *Ann. pharm. franç.*, **5,** 59(1947).
(11) Gillham, R. W., and Tomlinson, J. E., *Pharm. J.*, **162,** 472(1949).
(12) Shelton, R. S., and Magid, L., U. S. pat. 2,241,331 (1941).
(13) Ward, W. C., THIS JOURNAL, **39,** 265(1950).
(14) Gross, H. M., and Becker, C. H., *ibid.*, **41,** 157(1952).
(15) Manz, E., *Süddeut. Apoth. Zig.*, **90,** 321(1950).

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal, and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Norepinephrine
Benzoyl-L-argininamide
Ergothioneine
Amoidin
Methionic acid
D-Mannosamine hydrochloride
o-Pyrocatechuic acid
D-Thyroxin
Hydroxytetronic acid
Reductone
Xyluronic acid
Camphenilene
Xanthotoxin
Adamantane
Morpholine nitrite
Phenazine methochloride
Ellagic acid
Triphenyl fulgide
D-Cysteine

A Method of Evaluating Local Anesthetics*,†

By WALTER L. MACK‡ and JOHN W. NELSON§

A method of evaluating local anesthetics, based on the response of albino rats to heat stimulus applied to the tail, is presented. Four local anesthetics were tested and the duration of anesthesia for a given dose was determined. A linear relationship was shown to exist between concentration and duration of anesthesia with procaine, Metycaine, and Nupercaine, but cocaine did not exhibit this same pattern.

THE high efficiency and general satisfaction of local anesthetic procedures today is the result of three great discoveries in this field and their subsequent exploitation. First, there was the isolation of cocaine from coca leaves by Albert Niemann in 1858. The second discovery was made by Carl Koller while working in conjunction with Sigmund Freud in Vienna. He noted the benumbing effect of cocaine on the tongue and recognized the potentialities of the drug for local anesthesia (1). The third major contribution was made by the Germans Einhorn and Braun who synthesized the drug novocaine. Braun also utilized the vasoconstrictor action of epinephrine to lessen the rate of absorption and prolong the time of action of cocaine, which made it safer and more efficient (2).

The thousands of compounds being investigated for local anesthetic action require a suitable pharmacological method for preliminary testing. The successful use of pain threshold determination in measuring the analgetic potency of drugs suggests it for evaluation of local anesthetics.

A method based on the response of rats to a heat stimulus applied to the tail was proposed by Davies, *et al.* (3), for the evaluation of analgesic activity. Tye and Christensen (4) determined analgesic potency by using the Hardy-Wolff-Goodell pain threshold apparatus to measure the response of rats to heat stimulus applied to the tail. An application of this apparatus to evaluate local anesthetics appeared favorable because the intensity of the stimulus can be controlled and its severity need not be great enough to cause tissue damage before pain is elicted.

* Received August 22, 1952, from the College of Pharmacy, The Ohio State University, Columbus.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.
† Abstract of a thesis presented to the Graduate School of The Ohio State University by Walter L. Mack in partial fulfillment of the requirements for the degree of Master of Science.
‡ Present address: Assistant Professor of Pharmacology, College of Pharmacy, University of Toledo, Toledo, Ohio.
§ Professor of Pharmacy, College of Pharmacy, The Ohio State University.

EXPERIMENTAL

The apparatus employed was the Hardy-Wolff-Goodell pain threshold apparatus modified by Tye, consisting of a 500-watt lamp, the light beam focused to an aperture by a condensing lens; a variac transformer to control the intensity; a sensitive voltmeter to indicate the intensity of the radiation in millicalories/sec./cm.^{2}; an electronically controlled shutter; and a timer. A coil of copper tubing through which cold water was passed was wound around the length of the metal tube between the lens and the aperture to prevent heating of the tube by conduction. A piece of asbestos tape was placed beneath the shutter which cut off the heat stimulus, thus absorbing disturbing noises.

The albino rat, after a short training period, was a satisfactory laboratory animal for this work; its normal pain threshold is similar to that of humans for a three-second exposure to the stimulus, and the rat tail is sensitive enough to give an easily discernible response.

The pain threshold was previously determined by exposure to the stimulus at frequent intervals over a period of several weeks.

All animals used were white females, C. F. Wistar strain, weighing 150–250 Gm. Feeding and storage were kept uniform. Laboratory noises were kept at a minimum during the actual working of the experiment.

PROCEDURE

The pain threshold for each animal was determined by blackening its tail with India ink and allowing it to dry. The rat was then placed in a holder and injected subcutaneously with normal saline in the blackened portion of the tail; the injection area was exposed at five-minute intervals to stimuli of 400, 350, and 300 millicalories, and gradations of 10 millicalories lower until the rat failed to move its tail. A slight twitch or quiver was regarded as a negative response, a jerk or removal of the tail as a positive response. The lowest number of millicalories to elicit a response after repeated stimulation at five-minute intervals was taken as the threshold of the animal, and was repeated for several weeks until constant.

Following this determination, each local anesthetic to be tested was injected subcutaneously in the rat's tail at a marked point approximately two inches from the body of the animal.

Four local anesthetics were used in this work: procaine (Novocaine), piperocaine hydrochloride (Metycaine), cocaine (benzoyl methyl ecgonine), and dibucaine hydrochloride (Nupercaine), in concentrations of 0.05, 0.1, 0.2, and 0.5%.

Determination of Duration of Anesthesia.—In this experiment a comparison of the duration of anesthesia of the four concentrations of each of the four anesthetics was made. Female rats in groups of twelve were injected subcutaneously with 0.1

ml. each of the test preparation and the duration of anesthesia was determined by exposing the blackened portion of the tail to the threshold stimulus applied at five-minute intervals until a positive response was elicited. The results are shown in Table I.

TABLE I.—DURATION OF ANESTHESIA WITH 0.1 ML. DOSE, MIN.[a]

Concn., %	0.05	0.1	0.2	0.5
		Procaine		
M	5.0	9.1	16.6	36.6
AD	1.6	2.8	2.4	7.2
SD	3.0	2.9	3.1	9.7
		Metycaine		
M	14.5	31.2	54.5	68.7
AD	0.4	1.8	4.3	8.5
SD	1.3	2.2	6.0	14.5
		Cocaine		
M	26.2	29.5	30.0	101.2
AD	3.9	11.8	11.6	23.9
SD	6.0	14.2	16.9	29.1
		Nupercaine		
M	29.5	47.0	69.1	126.6
AD	1.9	5.0	14.7	9.1
SD	3.2	6.5	20.9	12.9

[a] M = Mean of 12 animals. AD = Average deviation from the mean. SD = Standard Deviation.

A similar set of experiments was made with a standard dose of 0.2 ml.; results are given in Table II.

TABLE II.—DURATION OF ANESTHESIA WITH 0.2 ML. DOSE, MIN.[a]

Concn., %	0.5	0.1	0.2	0.5
		Procaine		
M	13.3	13.3	30.4	64.1
AD	6.9	2.2	3.0	18.6
SD	5.7	2.3	5.4	24.7
		Metycaine		
M	33.7	57.9	76.2	120.0
AD	3.1	11.5	10.2	7.5
SD	3.7	13.9	14.9	14.9
		Cocaine		
M	34.16	47.91	50.41	160.0
AD	7.2	11.5	15.9	5.0
SD	11.4	17.6	20.3	7.3
		Nupercaine		
M	46.2	52.5	87.5	185.0
AD	5.4	3.4	15.0	24.1
SD	9.5	4.5	21.8	46.6

[a] M = Mean of 12 animals. AD = Average deviation from the mean. SD = Standard Deviation.

DISCUSSION

In current methods of evaluating local anesthetics, cocaine is usually employed as the reference standard. In the proposed method, another local anesthetic, such as procaine, should be used. Cocaine does not produce a pattern similar to that of the others; also, the fact that it possesses vasoconstrictor properties, whereas the others do not, introduces another factor into the procedure. Bülbring and Wajda (5) in making biological comparisons of local anesthetics showed that the lines relating concentration to the anesthetic effect of procaine and Nupercaine are parallel to one another but not to cocaine. They too suggested the advisability of using procaine rather than cocaine as the assay standard; if cocaine is used, however, epinephrine must be added because of its vasoconstrictor activity.

When the dose was increased to 0.2 ml., a strikingly similar pattern of effect was produced. Though only four concentrations of anesthetics were used, the linear relationship (Figs. 1 and 2) establishes

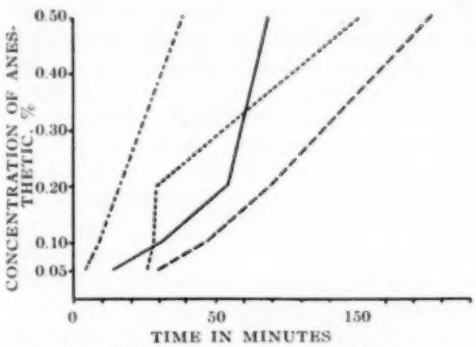


Fig. 1.—Duration of anesthesia with 0.1 ml. dosage. —·— Procaine. ——— Metycaine. - - - - Cocaine. —— Nupercaine.

the fact that the duration of anesthesia is dependent on the concentration of the anesthetic used for a given dose. Concentrations between 0.2 and 0.5% will be studied at a later date.

From the data accumulated it is evident that the Hardy-Wolff-Goodell pain threshold apparatus can be useful in evaluating local anesthetics; it produces a measurable sensation of pain, the stimulus is uniform and produces a uniform response, and the response has been shown to be reproduced in a number of animals with little or no variation. The

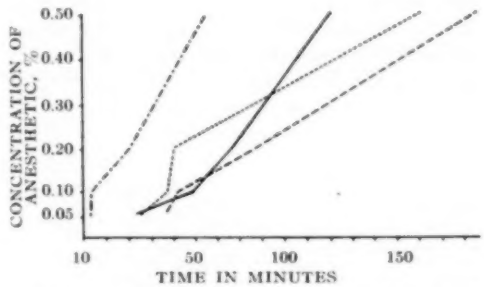


Fig. 2.—Duration of anesthesia with 0.2 ml. dosage. —·— Procaine. ——— Metycaine. - - - - Cocaine. —— Nupercaine.

method is not complicated; its use of small animals is economical and convenient.

CONCLUSIONS

1. Because of the successful use of the Hardy-Wolff-Goodell pain threshold apparatus in evaluating analgesics, this method was applied to the evaluation of local anesthetics.

2. A linear relationship was shown to exist between concentration and duration of anesthesia for a given dose with procaine, Metycaine, and Nupercaine.

3. This same relationship was shown to hold true at an increased dose volume.

4. Cocaine does not give the same pattern as the other anesthetics, probably due to its vasoconstrictor action.

5. This method gives evidence of being reasonably rapid, convenient, and reliable for the evaluation of local anesthetics, using procaine as a reference standard.

REFERENCES

(1) Alexander, F. A. C., *Science Digest*, **7**, 82(1940).
(2) Hertzler, A. E., "Technic of Local Anesthesia," C. V. Mosby, St. Louis, 1947, pp. 17–28.
(3) Davies, O. L., Raventos, J., and Walpole, A. L., *Brit. J. Pharmacol.*, **1**, 255(1946).
(4) Tye, A., and Christensen, B. V., THIS JOURNAL, **40**, 404(1951).
(5) Bülbring, E., and Wajda, I., *J. Pharmacol., Exptl. Therap.*, **85**, 78(1945).

A New Technique for the Evaluation of Ointment Bases*

By WALTER L. MACK,† JOHN W. NELSON,‡ and EARL P. GUTH§

By making use of the Hardy-Wolff-Goodell pain threshold apparatus, it is possible to show a significant difference in the relative efficiency of release of Nupercaine from different ointment bases. Simple ointment U. S. P., hydrophilic ointment U. S. P., and a bentonite ointment base are compared.

A TECHNIQUE for the evaluation of ointment bases using the Hardy-Wolff-Goodell pain threshold apparatus was suggested by the work of Christensen and Tye (1, 2) in their work on the evaluation of analgetics. The possibility of using this apparatus was further stimulated by the work of Nelson and Mack (3) in their studies concerning the development and standardization of a method for the evaluation of local anesthetics using this special apparatus. This report deals with the results of using this Hardy-Wolff-Goodell apparatus in measuring the relative release and absorption of Nupercaine from four different ointment bases. The tail of the albino rat served as the test organ.

* Received August 22, 1952, from the College of Pharmacy, The Ohio State University, Columbus.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.
† Professor of Pharmacology, College of Pharmacy, University of Toledo, Toledo, Ohio.
‡ Professor of Pharmacology, College of Pharmacy, The Ohio State University.
§ Professor of Pharmacy, College of Pharmacy, The Ohio State University.

EXPERIMENTAL

The pain threshold of each animal was determined as follows: the tail of the rat was immersed in a 1% water solution of Nupercaine for thirty minutes, then taken out, and the excess solution removed by gentle dabbing with cotton. The tail was then coated with India ink. When the ink had dried, the animal's tail was subjected to 400 millicalories of heat in the Hardy-Wolff-Goodell pain threshold apparatus at one-minute intervals until a positive reaction to the stimulus was noted. The pain threshold of each animal was checked before each experiment. The average duration of anesthesia was eight minutes.

One per cent of Nupercaine was incorporated into each of the following bases: simple ointment U. S. P., hydrophilic ointment U. S. P., and bentonite ointment (4). A commercial product[1] was also tested. Results are shown in Table I.

The ointments were applied to the rat's tail, allowed to remain for one hour, and removed. The tail was then prepared with India ink and subjected to testing in the pain threshold apparatus.

The results of the study show that simple ointment U. S. P., when used as a base for Nupercaine, does not release the anesthetic, since no evidence of anesthesia could be found after the ointment was removed from the tail. The test did give evidence that the anesthetic was released from hydrophilic ointment, bentonite ointment, and the commercial product. The bentonite base appeared to be the most efficient for the release of Nupercaine.

[1] Nupercainal—Ciba.

CONCLUSIONS

1. The Hardy-Wolff-Goodell pain threshold apparatus can be used to test the release of anesthetics from ointment bases.

2. The tail of the albino rat is a convenient testing organ for ointments.

3. There is a measurable difference in the efficiency of release of anesthetics from different ointment bases.

TABLE I.—EXPERIMENTAL DATA

Ointment	Time of Anesthesia, Min.	No. of Animals Used
Simple ointment	0	4
Hydrophilic ointment	0	4
Bentonite ointment	0	4
Simple ointment + 1% Nupercaine	0	4
Hydrophilic ointment + 1 % Nupercaine	10	5
Bentonite base + 1% Nupercaine	15–20	8

REFERENCES

(1) Christensen, B. V., and Tye, A., THIS JOURNAL, **40**, 404(1951).
(2) Christensen, B. V., and Tye, A., *ibid.*, **41**, 75(1952).
(3) Mack, W. L., and Nelson, J. W., *ibid.*, **42**, 101(1953).
(4) Darlington, R. C., and Guth, E. P., J. AM. PHARM. ASSOC., *Pract. Pharm. Ed.*, **11**, 82(1950).

A Study of the Iron-Gentisate Reaction and Its Application*

By JOHN G. WAGNER†

A hitherto unreported reaction of gentisic acid with ferric ion is studied. Optimum conditions for the development of the iron gentisate color are determined. An application of the reaction is indicated.

GENTISIC ACID (2,5-dihydroxybenzoic acid), a biological oxidation product of salicyclic acid, possesses a therapeutically useful antirheumatic activity (1). The most comprehensive physicochemical data for gentisic acid was reported by Osol and Kleckner (2). In the course of their study, a reaction of gentisic acid with molybdophosphotungstate T. S. was observed and studied, but no details regarding the use of this reaction for the colorimetric determination of gentisic acid were given.

Recently, Pankratz and Bandelin (3) reported a systematic and comprehensive study of the optimum conditions for the well-known iron-salicylate reaction. Their assay method for salicylate ion has been adapted by this author for use with a Cenco-Sheard-Sanford Photelometer, utilizing a green filter, and satisfactory results were achieved.

Due to the fact that gentisic acid has the same grouping (a phenolic hydroxyl group *ortho* to a carboxyl group on a benzene ring) the author studied the reaction between gentisic acid and ferric ion. When a ferric nitrate solution is added to a dilute solution of gentisic acid, a blue color develops immediately, but disappears in five to ten seconds, leaving a yellow solution. The intensity of color of the yellow solution, at the low concentrations employed, varies only slowly with time.

An investigation of the optimum conditions for the reaction and the possible use of the reaction in the determination of gentisic acid and its salts was made.

EXPERIMENTAL

Purification of Gentisic Acid

A commercial grade of gentisic acid (Delta-Chemical Works) was purified according to the procedure of Osol and Kleckner (2).

Reagents

(*a*) Ferric nitrate reagent: 1.0 Gm. of $Fe(NO_3)_3 \cdot 9H_2O$ was dissolved in a sufficient quantity of exactly 1% nitric acid to make 100 ml. of solution.

(*b*) Concentrated solution of gentisic acid: 250 mg. of purified gentisic acid was dissolved in sufficient distilled water to make 100 ml. of solution.

(*c*) Dilute solution of gentisic acid: exactly 10 ml. of the concentrated solution of gentisic acid was pipetted into a 1-L. volumetric flask and diluted to volume with distilled water. Each milliliter of this solution contained 25 μg. of gentisic acid.

* Received September 11, 1952, from the College of Pharmacy, The Ohio State University, Columbus.
† Assistant Professor, College of Pharmacy, The Ohio State University.

Determination of the Absorption Curve of the Iron-Gentisate Complex

A colored iron-gentisate solution, containing the equivalent of 12.5 μg. of gentisic acid, was prepared by mixing 25.0 ml. of dilute gentisic acid solution, 20.0 ml. of distilled water, and 5.0 ml. of ferric nitrate reagent. Absorption measurements were made in a Beckman Model DU Spectrophotometer. The blank was prepared from 45.0 ml. of water and 5.0 ml. of ferric nitrate reagent. Corrections were applied for the differences in the cells. The absorbancy of the ferric-gentisate solution between 360 and 600 mμ was determined; maximum absorption occurs at 420 mμ. A plot of absorbancy (E) versus wave length for this solution is shown in Fig. 1.

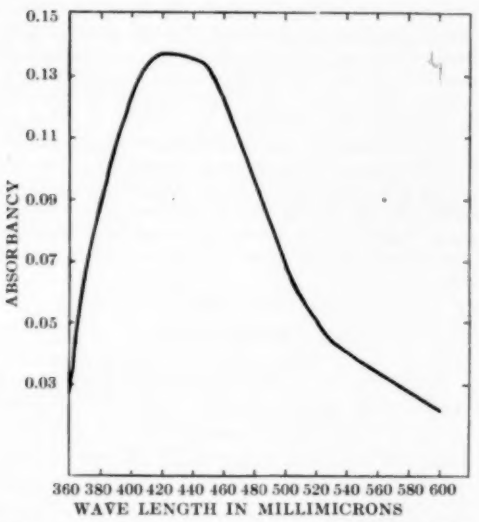


Fig. 1.—Absorption spectrum of iron-gentisate solution.

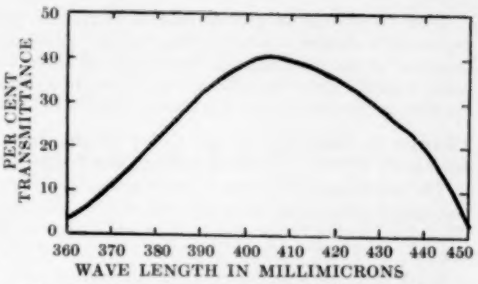


Fig. 2.—Transmittancy range of the blue filter.

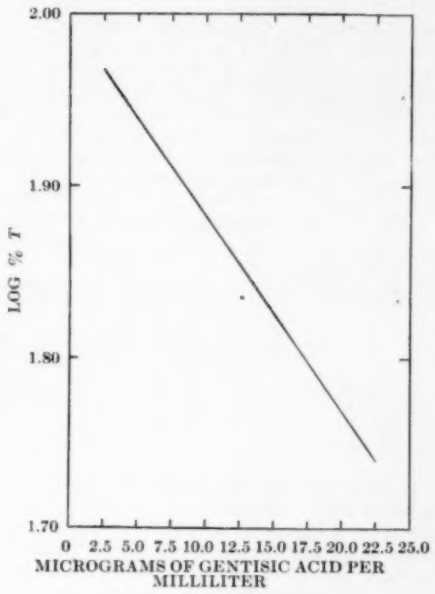


Fig. 3.—Standard curve.

Preparation of a Standard Curve

Choice of Filter.—A blue filter having maximum transmission in the range 400–450 mμ would be suitable for use with a photeleometer. The author employed a cobalt blue filter with maximum transmission at 405 mμ. The transmission range of the filter was determined in a Beckman DU Spectrophotometer. The instrument was set at 100% T with the open slit at each wave length; the filter was then placed in the light path and the % T was read. The transmittancy of the filter between 360 and 450 mμ is shown in Fig. 2.

Calibration Procedure.—Aliquot portions of the dilute gentisic acid solution ranging from 5 to 45 ml. in 5-ml. increments were measured by burette. Where necessary, distilled water was measured by burette to make each solution up to 45 ml. Five milliliters of ferric nitrate reagent was added to each solution. The per cent transmittance of each solution was determined on a Cenco-Sheard-Sanford Photelometer, using the cobalt blue filter described above, within three to eight minutes after adding the reagent. By plotting the concentration of gentisic acid against log % T, a straight line was obtained which was in conformity with Beer's law. The calibration graph is shown in Fig. 3.

Change in Intensity of the Color of the Iron-Gentisate Solution with Time

The color produced in acid solution changes very slowly in intensity with time in the range of concentrations employed. As the concentration decreases, the rate of change of intensity of color with time decreases. The rate of change in intensity of the color of an iron-gentisate solution at the wave length of maximum absorption (420 mμ) is shown in Table I.

TABLE I

Absorbancy (E)	Time, Min.
0.165	3–11
0.163	15
0.162	19
0.158	36
0.157	51

Over the range of concentrations employed, no perceptible change in the reading for each solution occurred if the readings were taken within three to eight minutes after the addition of the ferric nitrate reagent to the gentisic acid solution.

Change in Intensity of the Color of the Iron-Gentisate Solutions with the *p*H of the Solutions

The intensity of the color of a given iron-gentisate solution is dependent upon the *p*H of the solution. This was shown by preparing a dilute solution of gentisic acid and adding to 45-ml. aliquots of the solution 5 ml. of 1% $Fe(NO_3)_3 \cdot 9H_2O$ solutions containing different concentrations of nitric acid. The *p*H of each solution and of the corresponding blank was determined on a Beckman Model G *p*H meter with external electrodes. The % *T* of each solution was determined as described above under *calibration procedure*. The *p*H of each blank was found to be the same as the *p*H of the corresponding colored solution. The ferric nitrate reagents containing 0.47% or more of nitric acid were colorless, but those containing 0.1% and 0.01% of nitric acid were yellow, due to the hydrolysis of the ferric ion. The data obtained are shown in Table II.

TABLE II

Concn. of HNO_3 in Ferric Nitrate Reagent, %	% *T* of Colored Soln.	*p*H of Colored Soln.
10	66.0	0.78
5	60.0	1.09
1	56.5	1.75
0.47	56.0	2.00
0.1	57.5	2.42
0.01	58.0	2.62

It can be seen that maximum color intensity occurs at about *p*H 2. However, the intensity at *p*H 1.75 differs little from that at *p*H 2. From the standpoint of stability of the ferric nitrate reagent and the fact that the colorimetric determination of salicylates (3) requires the same reagent, the ferric nitrate reagent containing 1% HNO_3 was used in preparing the standard curve.

Assay of Gentisic Acid (and Its Salts)

In order to assay an unknown sample of gentisic acid, it is necessary only to prepare a dilute solution of the gentisic acid of approximately the same strength as that used in the preparation of the standard curve. The procedure then follows the same pattern as described under *calibration procedure*. Once the % *T* of the colored solution is obtained and the concentration of gentisic acid in the colored solution is read from the calibration graph, calculation of the per cent of gentisic acid in the original unknown sample involves only consideration of the dilution factors.

An example to illustrate the procedure is given. A sample of commercial gentisic acid attained constant weight after being heated at 105° for three and one-half hours. A portion of 0.3191 Gm. was transferred to a 100-ml. volumetric flask and dissolved in distilled water, and sufficient water was added to bring to volume. An aliquot of 10 ml. of this solution was diluted to exactly 1 L. An aliquot of 25 ml. of this dilute solution, 20 ml. of water, and 5 ml. of ferric nitrate reagent gave a colored solution for which % $T = 67$, corresponding to a concentration of 15 μg. of gentisic acid per ml. in the colored solution. The per cent of gentisic acid in the original weighed sample is given by: $2 \times 15.0 \times \frac{10}{1000} \times \frac{100}{0.3191} = 94.01\%$ of gentisic acid. Aliquots of 35 ml. and 20 ml. of the dilute solution gave values of 94.95% and 93.71% of gentisic acid respectively. The mean concentration of gentisic acid was 94.2%. The reproducibility is within ±1% of the mean. Good precision is also illustrated by the fact that under the conditions outlined above, a standard curve with the same slope was reproduced three times when three different samples of purified gentisic acid were utilized.

The ammonium and sodium salts of gentisic acid could be assayed by the same procedure, since the small concentrations of the salts involved would not affect the *p*H of the colored solutions to any significant degree. For utmost accuracy, a calibration curve could be constructed, using a pure sample of the salt.

SUMMARY

Treatment of a dilute solution of gentisic acid with a ferric nitrate solution yields a blue-colored solution which rapidly changes to a yellow solution, the intensity of which, at the low concentrations employed, varies only slowly with time. The maximum absorption of the yellow solution occurs at 420 mμ, and solutions of the compound conform to Beer's law. The intensity of the color of a given iron-gentisate solution is dependent upon the *p*H of the solution. The most favorable range is from *p*H 1.75 to 2.0. A standard curve in the range 2.5 to 22.5 μg. of gentisic acid per ml. of colored solution is readily reproducible, using a Cenco-Sheard-Sanford Photelometer with a cobalt blue filter having maximum transmission at 405 mμ. The value of the reaction for the assay of gentisic acid (and its salts) is indicated.

REFERENCES

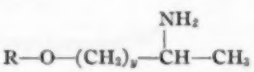
(1) Meyer, K., and Ragan, C., *Science*, **108**, 281(1948).
(2) Osol, A., and Kleckner, L. J., THIS JOURNAL, **41** 306(1952).
(3) Pankrantz, R. E., and Bandelin, F. J., *ibid.*, **41**, 267 (1952).

A Pharmacological Study of Certain Alkoxyalkylamines*

By TOM S. MIYA and L. D. EDWARDS†

A series of alkoxyalkylamines has been tested for vasoconstrictor activity. The most active of the series, Compound D-9 [2-amino-3-(2-methylpropoxy)propane], is compared pharmacologically with Tuamine and Forthane.

SINCE the isolation of epinephrine and the investigations of the active principle ephedrine from the Chinese herb, Ma Huang (1–3), sympathomimetic amines have been studied extensively. The demonstration of the pressor activity of aliphatic amines (4) has directed investigations to this type of compound. Sutton and Data (5) synthesized a series of alkoxyalkylamines thought to possess vasconstrictor properties and thus to be useful for the relief of nasal congestion, of the general formula:



in which R is an alkyl group and y is 1 to 3 inclusive. The following compounds were made available to this laboratory for study: the methoxy, ethoxy, propoxy, butoxy, 2-methylpropoxy, and pentoxy derivatives of 2-aminopropane; the methoxy, ethoxy, and propoxy derivatives of 2-aminobutane; and the ethoxy and propoxy derivatives of 2-aminopentane.

After screening the entire group for pressor activity on the pithed rat blood pressure, the most active compound of the series was further compared with 2-aminoheptane sulfate (Tuamine sulfate)[1] and 2-amino-4-methylhexane sulfate (Forthane sulfate),[1] two aliphatic amines with nasal decongestant properties which have enjoyed wide commercial acceptance in the past decade. All compounds were tested in the form of sulfate.

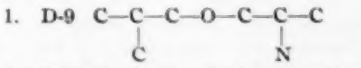
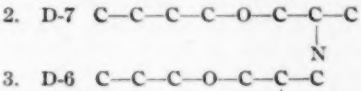
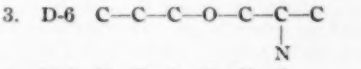
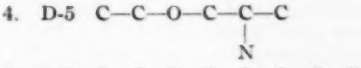
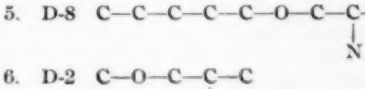
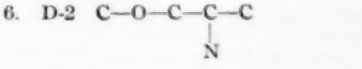
EXPERIMENTAL

Pressor Activity.—White rats, 150–250 Gm., were anesthetized and pithed according to the method of Shipley and Tilden (6). A respirator for small animals as described by Edwards and Miya (7) was employed. The blood pressure was taken from the carotid artery with a small bore (5 mm.) mercury manometer with polyethylene tubing bridging the animal to the manometer. Sodium thiosulfate (20%), fortified with heparin in the region of the cannula, was employed as the anticlotting agent. Two or more compounds were injected in turn into the iliac vein, allowing sufficient time for recovery after each injection. The peak rises in blood pressure determined on a minimum of three animals were used as the criterion of activity. The compounds are listed in Table I in decreasing order of activity in their respective groups.

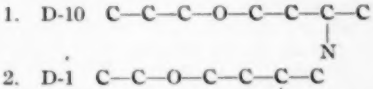
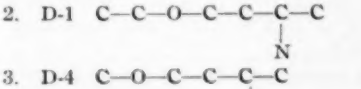
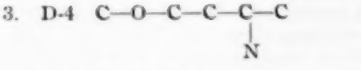
The screening tests indicate that the pressor activity increases with molecular weight up to a cer-

TABLE I.—COMPOUNDS IN DECREASING ORDER OF ACTIVITY

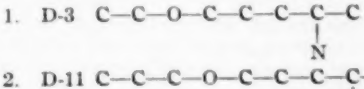
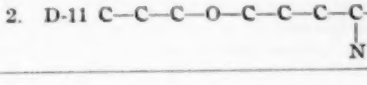
Derivatives of 2-Aminopropane:

1. D-9 
2. D-7 
3. D-6 
4. D-5 
5. D-8 
6. D-2 

Derivatives of 2-Aminobutane:

1. D-10 
2. D-1 
3. D-4 

Derivatives of 2-Aminopentane:

1. D-3 
2. D-11 

* Received August 22, 1952, from Purdue University, School of Pharmacy, Lafayette, Ind.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.

† Professor of Pharmacology, Purdue University, School of Pharmacy.

[1] Kindly supplied by Dr. K. K. Chen, Eli Lilly and Co.

tain point, e.g., in the derivatives of 2-aminopropane, from 2-amino-3-methoxypropane to 2-amino-3-butoxypropane inclusive; further increase in molecular weight appears to decrease pressor activity. The most active compound, an isomer of 2-amino-3-butoxypropane, is 2-amino-3-(2-methylpropoxy)-propane (Compound D-9); next in order are 2-amino-4-propoxybutane (Compound D-10) and 2-amino-3-butoxypropane (Compound D-7) which appear to possess approximately one-half the activity of Compound D-9. The compound with the least activity is 2-amino-3-methoxypropane (Compound D-2) which shows a transitory drop in blood pressure followed by an insignificant rise. Thus optimum activity is observed in a seven-carbon molecule. Swanson and Chen (4) have also reported that the optimum number of carbon atoms for pressor activity in aliphatic amines appears to be seven.

Compound D-9, Tuamine, and Forthane were later evaluated by the pithed rat blood pressure using epinephrine hydrochloride as the standard. The mean microgram epinephrine equivalent and S. E. of a 1-mg. dose of each compound is as follows: Tuamine, 0.353 ± 0.025; Forthane, 0.139 ± 0.007; and Compound D-9, 0.274 ± 0.024. Ten, eight, and twelve animals, respectively, were employed in the determinations. It has been shown that Forthane is more active than Tuamine in the dog (4).

Analeptic Activity.—Analeptic properties of Tuamine, Forthane, and Compound D-9 were determined, using pentobarbital sodium-induced narcosis (65 mg./Kg.) in white Swiss male mice, by a method patterned after that of Marsh and Herring (8). The mice, ranging in weight from 20 to 35 Gm., were fasted for twelve hours. In order to minimize variations, all tests were performed on a single day. The narcotizing agent was given intraperitoneally and fifteen minutes later a 20-mg./Kg. dose of one of the three amines was administered via the same route. Control mice received a corresponding volume of saline. The duration of narcosis was taken as the elapsed time between injection of the narcotizing agent and regaining of the righting reflex. Ten animals each were employed for the controls and for Tuamine; twenty animals each were employed for Compound D-9 and Forthane.

At the 5% confidence level, Tuamine and Forthane were observed to possess analeptic activity against pentobarbital sodium-induced narcosis while Compound D-9 did not. The mean durations of narcosis and S. E. in minutes were as follows: Control, 61.4 ± 4.02; Tuamine, 41.9 ± 2.14; Forthane, 49.2 ± 49.2 ± 5.60; and Compound D-9, 64.2 ± 6.70.

Nasal Volume and Blood Pressure Responses in Dogs.—Medium-sized dogs, 5.8 to 10.0 Kg., were anesthetized intramuscularly with 35 mg./Kg. of pentobarbital sodium and atropinized with 1.0 mg./Kg. Jackson's method (9) was followed for the recording of the nasal volume. The blood pressure was recorded by direct cannulation of the carotid artery, and injections were made into the femoral vein. Five animals were employed in these tests. Doses of 10–20 mg./dog of Compound D-9 and 5–10 mg./dog of Tuamine and Forthane produced an appreciable increase in nasal volume. Blood pressure responses indicate that the pressor activity of Compound D-9 is approximately one-half that of Tuamine and Forthane.

Nictitating Membrane Response in the Cat.—Cats were anesthetized with a mixture of 25% urethan and 3% pentobarbital sodium (1 mg./Kg. intraperitoneally). Blood pressure and nictitating membrane recordings were made simultaneously. In certain cases, *dl*-lupanine, a ganglionic blocking agent (10), was administered in doses of 2–10 mg./Kg. just before the injection of the amines. Both Compound D-9 and Tuamine in doses ranging from 5 to 10 mg. per cat, administered intravenously, consistently retracted the membrane before and after the administration of *dl*-lupanine. This effect was accompanied by a sharp elevation of the blood pressure (see Fig. 1), in the seven animals employed. Thus, the action of these compounds on this organ is peripheral to the ganglion. Charlier (11) has reported the retraction of the nictitating membrane with Forthane.

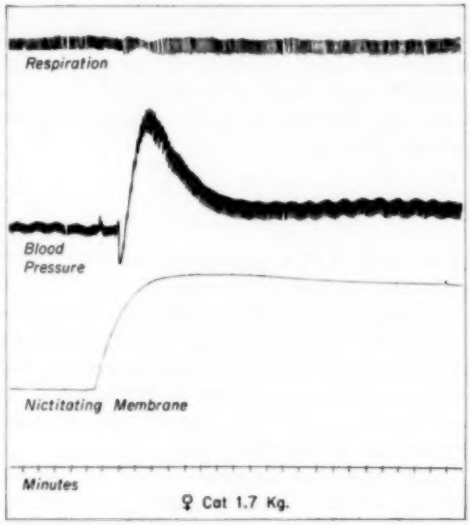


Fig. 1.—Effect of 10 mg. of Compound D-9 on the blood pressure and nictitating membrane of the cat.

Rat Hind Leg Perfusion.—Albino rats, 150–200 Gm., of both sexes, were employed for the tests. The skin and muscles of the hind legs and tail of each rat were perfused according to the method described by Hyman (12), with slight variations. The anterior portion was not cut away nor was a "running weight" of the preparation observed. Warm, oxygenated Locke Ringer's solution at a pressure of ca. 35 mm. of Hg was employed as the perfusate. Normal perfusion rate in drops per minute was taken for five minutes; the compounds in 2-mg. doses were introduced just proximal to the aortic cannula and the drops per minute were averaged for the subsequent five minutes. The average decrease in rate of flow ranged from 16.8 to 19.4% in the 24 trials. The activity ratios were 1.0, 1.09, and 1.15 for Tuamine, Forthane, and Compound D-9, respectively. Thus the activities of the compounds with this preparation are approximately equal.

Frog Hind Leg Perfusion.—The hind legs of frogs (*R. pipiens*) were perfused by the method of Trendelenburg (13). The amines were introduced as described above for the rat hind leg perfusion in 2-mg. doses. The average decrease in rate of flow ranged from 19.0 to 33.6% in the 24 trials. The activity ratios were 1.0, 0.82, and 1.45 for Tuamine, Compound D-9, and Forthane, respectively.

Frog Heart Perfusion in Situ.—Frog (*R. pipiens*) hearts were perfused *in situ* with Sollmann and Chu perfusate (14), employing a Greene cannula. The amines were administered via the standpipe in doses ranging from 0.25 to 3.0 mg. Myocardial depression produced with all three drugs was most pronounced with Tuamine and least with Compound D-9. Smaller doses failed to stimulate the hearts. Epinephrine hydrochloride (0.5 μg.) consistently produced positive inotropic and chronotropic effects.

Langendorff Hearts.—The hearts of 15 rats, 10 rabbits, 6 guinea pigs, and 4 dogs were perfused with warm (38°) oxygenated Locke Ringer's solution at a pressure of ca. 30 mm. of Hg for rat, guinea pig, and rabbit hearts, and ca. 50 mm. Hg for dog hearts. Double distilled (glass) water was used to prepare the perfusate. Doses of 0.625 to 1.0 mg. were injected just proximal to the aortic cannula in the rat and guinea pig, 0.75 to 1.0 mg. in the rabbit, and 0.0625 to 0.2 mg. in the dog. In the rat, guinea pig, and rabbit hearts, a slight decrease (1–2%) in coronary flow was observed. Only in the dog heart was an appreciable increase in coronary flow observed (10-15%). All three amines were qualitatively identical in a given species. In each species, Tuamine had the greatest activity, followed by Forthane and then Compound D-9. All three compounds produced irreversible myocardial depression at higher dosage levels. At lower dosages, there was depression followed by stimulation in the rat, guinea pig, and rabbit hearts, and stimulation without primary depression in the dog hearts.

Pressor Responses after Adrenolytic Agents.—Dibenamine hydrochloride,[2] 20 mg./Kg., administered intraperitoneally, and ergotoxine ethanesulfonate, 0.5 mg./Kg., administered intravenously, which reversed the pressor action of 0.5 μg. of epinephrine hydrochloride, did not appreciably alter the pressor response to Tuamine, Forthane, and Compound D-9 in the anesthetized white rat.

Inhibition of Acetylcholine-induced Contractions of the Rat Uterus.—The method described by Burns (15) was employed. Acetylcholine bromide, 1.2 to 8.0 μg./30-ml. bath, was employed to induce contractions. Tuamine was twice as active as Forthane in inhibiting acetylcholine-induced contractions. Compound D-9, in doses of 4–20 mg., failed to inhibit the contractions.

Guinea-Pig Lung Perfusion.—Guinea-pig lungs were perfused by the method of Tainter and Pedden (16). Doses of 3 mg. of Tuamine, Forthane, and Compound D-9, injected in six lungs just proximal to the tracheal cannula, reduced the perfusion rate 7.5 to 19.0%. Control injections with the perfusate did not alter the rate.

It has been reported by Neidle, *et al.* (17), that Tuamine restricts the flow through the isolated perfused guinea-pig lungs, and that ephedrine, which is known to relieve bronchial asthma, has the same effect.

Effects on Hog and Cattle Carotid Artery Spirals.—The method described by Lewis and Koessler (18) was employed. Hog and cattle carotid arteries, three hours to five days of age, consistently responded, by contraction, to 1:15,000 (final concentration) of Tuamine and Compound D-9. The latter appears to be more active. Forthane failed to evoke a response even in twice the concentration. There were no observable concentration-response variations in the three-hour and five-day-old preparations, indicating a musculotropic action.

Irritation Tests.—Irritation tests were performed by instilling 1% solutions of the amines in the rabbit's eye and also by subcutaneous injections of 1% solutions into the rabbit's ear by the method of Kuna and Cuchie (19). No symptoms of irritation were observed in either case. No changes in corneal sensitivity or pupil size were apparent.

Acute Toxicity in Mice and Rats.—White Swiss mice, 18–30 Gm., and albino rats (Sprague-Dawley), 85–130 Gm., of both sexes, were employed for the determinations. All animals were fasted but received water *ad lib.* for twenty-four hours prior to use. The temperature was kept as constant as possible for all tests (28–33°). The animals were kept under observation in individual cages for twenty-four hours, deaths were counted, and the LD_{50} for each case was determined by DeBeer's method (20).

All deaths occurred with terminal clonic convulsions within one to three minuts after injection except for the Forthane-injected mice in which deaths were delayed up to four hours. Hyperexcitability, exophthalmos, micturation, and profuse salivation were observed in the surviving animals which received Tuamine and Forthane; the same symptoms to a much lesser degree occurred in those which received Compound D-9. The comparative data are presented in Table II. Compound D-9 is the least toxic in both species.

Effects of Prolonged Administration in Mice.—White Swiss mice received 25% subcutaneously of the intravenous LD_{50} of Compound D-9, over a period of 32 days. No symptoms of toxicity or gross pathology of the spleen, liver, lung, heart, kidney, or intestines were observed. Histological examinations of the six organs gave no indications of pathological changes.

Miscellaneous.—The jejunal-ileal segments of rats and of guinea pigs responded by tonus rise to 1:6,000–1:120,000 (final concentration) of Tuamine, Forthane, and Compound D-9 even after atropinization (1:100,000). Compound D-9 elicited inconsistent responses when used on the rat intestine.

Concentrations of 1:200,000 to 1:1,000,000 (final concentration) of all three amines inhibited the peristaltic intestinal movements of the rat and guinea pig as recorded by the method of Picchioni and Edwards (21).

Topical application of 1 and 2% solutions of the amines and intravenous injections of doses which produced an appreciable rise in blood pressure failed to give consistent responses on the rat meso-appendix preparation prepared by the method of Chambers and Zweifach (22).

[2] Kindly supplied by Smith, Kline & French Laboratories, Philadelphia, Pa.

TABLE II.—COMPARATIVE DATA OF ACUTE TOXICITY IN MICE AND RATS (INTRAVENOUS)[a]

Compound	LD_{50} for Mice, Mg./Kg.	Toxicity Ratio	LD_{50} for Rats, Mg./Kg.	Toxicity Ratio	Ratio of Rats/Mice
Tuamine	16.3 (92–109)[b]	1	47.3 (96–104)	1	2.89
Forthane	39.0 (96–104)	0.42	72.5 (97–103)	0.65	1.86
D-9	40.0 (93–107)	0.41	98.2 (98–102)	0.48	2.45

[a] 10 animals were used in each of six dose ranges for each compound.
[b] Limits of error, %.

DISCUSSION

This investigation indicates that 2-amino-3(2-methylpropoxy)propane (Compound D-9) shows some promise as a medicinal agent. Its activity on the pithed rat blood pressure is greater than that of Forthane and approaches that of Tuamine; it appears to stimulate the central nervous system the least. Compound D-9 is the most active of the three drugs on the carotid artery spirals of hog and cattle. The toxicity of this compound as determined in rats and mice is lower than that of either Tuamine or Forthane; however, systemic toxicity has less bearing on the suitability of a compound intended to be used topically.

SUMMARY

(1) A series of alkoxyalkylamines possessing vasopressor activity has been investigated.

(2) The most active of the series is 2-amino-3-(2-methylpropoxy)propane (Compound D-9). It appears to be 77.6 per cent as active as Tuamine and more active than Forthane by the pithed rat blood pressure method.

(3) Compound D-9 has no analeptic activity against pentobarbital-induced narcosis in mice; it shrinks the nasal mucosa of dogs, retracts the nictitating membrane of the cat after ganglionic blockade, restricts the flow through the isolated perfused hind legs of the frog and the rat, and constricts isolated carotid arteries of hog and cattle.

(4) Acute and "repeated-dose" toxicity data are presented.

(5) In view of the activity of Compound D-9, compounds of this type should be investigated further.

REFERENCES

(1) Chen, K. K., and Schmidt, C. F., *Proc. Soc. Exptl. Biol. Med.*, **21**, 351(1924).
(2) Chen, K. K., and Schmidt, C. F., *J. Pharmacol. Exptl. Therap.*, **24**, 339(1924).
(3) Chen, K. K., THIS JOURNAL, **14**, 189(1925).
(4) Swanson, E. E., and Chen, K. K., *J. Pharmacol. Exptl. Therap.*, **88**, 10(1946).
(5) Sutton, B. M., and Data, J. B., THIS JOURNAL, **41**, 328(1952).
(6) Shipley, R. E., and Tilden, J. H., *Proc. Soc. Exptl. Biol. Med.*, **64**, 453(1947).
(7) Edwards, L. D., and Miya, T. S., THIS JOURNAL, **39**, 701(1950).
(8) Marsh, D. F., and Herring, D. A., *J. Pharmacol. Exptl. Therap.*, **103**, 178(1951).
(9) Jackson, D. E., "Experimental Pharmacology and Materia Medica," 2nd ed., C. V. Mosby Co., St. Louis, 1939, p. 95.
(10) Bogner, R., Ph.D. Thesis, Purdue University (1952).
(11) Charlier, R., *Arch. intern. pharmacodynamie*, **83**, 573 (1950).
(12) Hyman, C., *Am. J. Physiol.*, **142**, 671(1944).
(13) Trendelenburg, P., *Arch. exptl. Path. Pharmakol.*, **63**, 165(1910).
(14) Sollman, T. H., and Hanzlik, P. J., "Fundamentals of Experimental Pharmacology," 2nd ed., J. W. Stacy, Inc., San Francisco, 1939, p. 295.
(15) Burns, J. H., "Biological Standardization," 2nd ed., Oxford University Press, New York, 1950, p. 222.
(16) Tainter, M. L., and Pedden, J. R., *J. Pharmacol. Exptl. Therap.*, **51**, 371(1934).
(17) Neidle, E. A., Gruber, C. M., and Copeland, J. E., *Arch. intern. pharmacodynamie*, **87**, 64(1951).
(18) Lewis, J. H., and Koessler, K. K., *Arch. Internal Med.*, **39**, 182(1927).
(19) Kuna, S., and Cuchie, F., THIS JOURNAL, **38**, 516(1949).
(20) DeBeer, E., *J. Pharmacol. Exptl. Therap.*, **85**, 1(1945).
(21) Picchioni, A. L., and Edwards, L. D., THIS JOURNAL, **41**, 289(1952).
(22) Chambers, R., and Zweifach, B. W., *Am. J. Anat.*, **75**, 173(1944).

Pharmacognostical Study of American Berberis*

By V. H. SIMONIAN† and H. W. YOUNGKEN, Sr.‡

A historical survey of the drug berberis and its various therapeutic uses is presented. A comparative and detailed study is given of *Berberis aquifolium, Berberis nervosa,* and *Berberis boliviana,* consisting of morphological description; gross-structure study of the overground portions and the roots and rhizomes; detailed histological study and descriptions, diagnostic histological elements, cell sizes, and cell-contents of powdered roots and rhizomes; and the compilation of a table of information helpful in establishing and detecting the botanical origin of powdered berberis. A complete histological study of the roots and rhizomes of *Berberis boliviana,* comparatively new in this country, is reported for the first time.

THE drug berberis has been official in the United States Pharmacopeia and the National Formulary several times in the past, but is one of the vaguely defined drugs. The National Formulary VII (1) defines it as consisting of the dried rhizomes and roots of species of the section *Mahonia* (Nuttall) De Candolle of the genus *Berberis* Linné (family *Berberidaceae*).

Although the genus *Berberis* is a very large one, fortunately the representative plants yielding the berberis of American commerce are usually limited to a few botanical sources. The rhizomes and roots of all these plants do not exhibit distinct morphological characteristics when viewed in the entire state, and the necessity of establishing distinguishing characteristics is apparent if one is to be able to identify the common varieties of the drug obtained from closely related botanical sources.

In order to determine such exact diagnostic standards, authentic samples of these drugs, as well as the entire growing plants yielding the drug, were obtained from Dr. H. W. Youngken, Jr. of the University of Washington, Seattle; from the Medicinal Plant Garden of the Massachusetts College of Pharmacy in Jamaica Plain, Mass.; and from S. B. Penick and Company of New York.

The botanical origins of the present day berberis of American commerce were established and confirmed to be: (*a*) *Berberis aquifolium* Pursh.; (*b*) *Berberis nervosa* Pursh.; and (*c*) *Berberis boliviana* Lechler. These three plants are easily distinguished from each other in their growing state, as may be seen from an examination of Fig. 1. It is obvious that the three plants are so dissimilar that they should not readily be confused.

* Received August 22, 1952, from the Massachusetts College of Pharmacy, Boston.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.
† Professor of Pharmacognosy, Duquesne University, Pittsburgh, Pa.
‡ Research Professor of Pharmacognosy, Massachusetts College of Pharmacy.

Berberis aquifolium and *Berberis nervosa* have similar habits and are woody shrubs with evergreen coriaceous leaves. They are native to the woods of the Pacific Northwest, growing from California to British Columbia. *Berberis boliviana* is a South American plant, common in Bolivia at an altitude of 12,000–14,000 feet (2).

DESCRIPTION OF THE LEAVES

Figure 2, sketch B, represents the leaves of *Berberis aquifolium* which are compound leaves imparipinnate, and of 5 to 7 leaflets. The leaflets are evergreen, glabrous, shiny dark-green above, and dull, light grayish-green below. Each leaflet is broadly lanceolate to ovate in shape, 5–10 cm. in length. The margin is strongly spinulose-dentate, the apex is acuminate-spinose, and the base is asymmetric, obtuse, and sagittate.

Berberis nervosa also has compound leaves, as seen in Fig. 2, sketch A. They are imparipinnate of 9 to 15 leaflets which are evergreen, glabrous, shiny, slightly grayish-green on the upper surface, and dull-green on the lower surface. Each leaflet is broadly lanceolate, 5–10 cm. in length, with the margin strongly spinulose-dentate and with well developed lateral veins. The apex is acuminate-spinose, and the base is obtuse and sagittate.

The leaves of *Berberis boliviana* as seen in Fig. 2, sketch C, are simple of about 1 to 1.5 cm. in length, arising in fascicles of 5 to 8 leaves; sessile, or on a very short, attenuated petiole. The lamina is tripartite, obovate, and cuneiform, each part ending with an acuminate spiny apex. The margin is callous, the base cuneate, and the venation reticulate.

USES OF BERBERIS

The medicinal uses of berberis in the crude form or as its most active constituent, berberine, have long been known. They are in considerable use today, most extensively in the treatment of tropical diseases including malaria and infections of *Leishmania* (3–7).

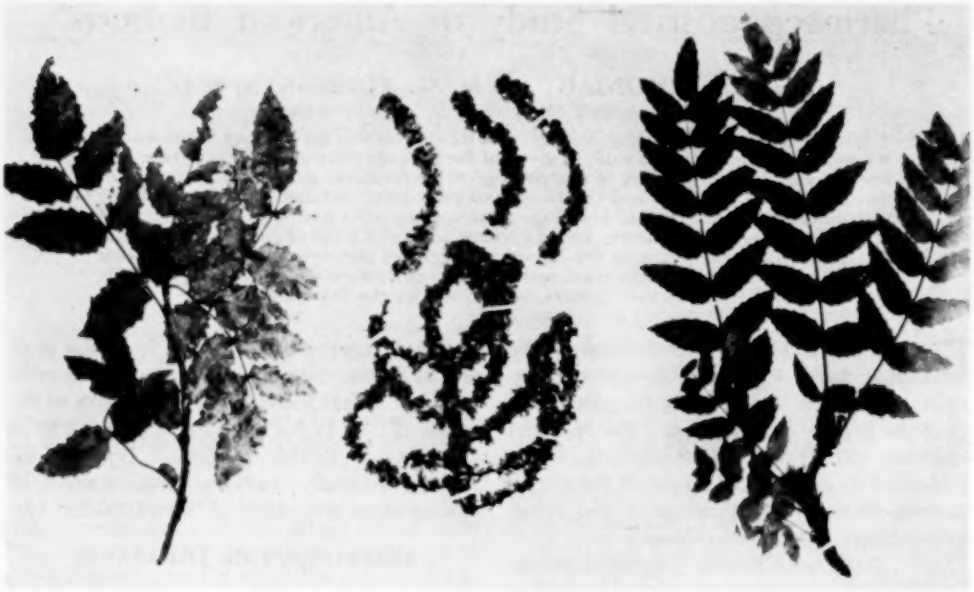


Fig. 1.—Photographs of authentic herbarium sheets of *Berberis aquifolium* (at left), *Berberis nervosa* (at right), and *Berberis boliviana* (in center).

A NEW SOURCE OF AMERICAN BERBERIS

Berberis aquifolium and *Berberis nervosa* have been reported in the literature as being sources of berberis of American commerce. In this paper *Berberis boliviana* is being reported for the first time as a source of commercial berberis. Fragmentary descriptions of berberis obtained from *Berberis aquifolium* exist in the National Formulary VII (1), "The Dispensatory of the United States of America" (8), Youngken's "Textbook of Pharmacognosy" (9), the British Pharmaceutical Codex (10), and in Short's comparative study of *Berberis aristata* (11). However, a survey of the literature reveals that no sufficient study has been made on the histology of the roots and rhizomes of *Berberis nervosa* and *Berberis boliviana*. This work was therefore undertaken to place on record a precise and complete histological study of the roots and rhizomes of *Berberis nervosa* and of *Berberis boliviana* which should serve to establish diagnostic characteristics for the drugs obtained.

EXPERIMENTAL

Sectioning.—All samples used in the preparation of sections were prepared by softening the entire pieces of the drug, about 2 cm. in length, in 48% hydrofluoric acid for a period of five weeks. Other less severe treatment did not yield a soft enough material to obtain sections satisfactory for permanent mounts. The softened material was then gradually and slowly dehydrated, and was imbedded in celloidin, giving blocks of appreciable size and of a proper consistency for sectioning on a sliding microtome.

Isolation of Elements.—Many methods for isolating the elements were tried; the most suitable was found to be Jeffrey's maceration method.

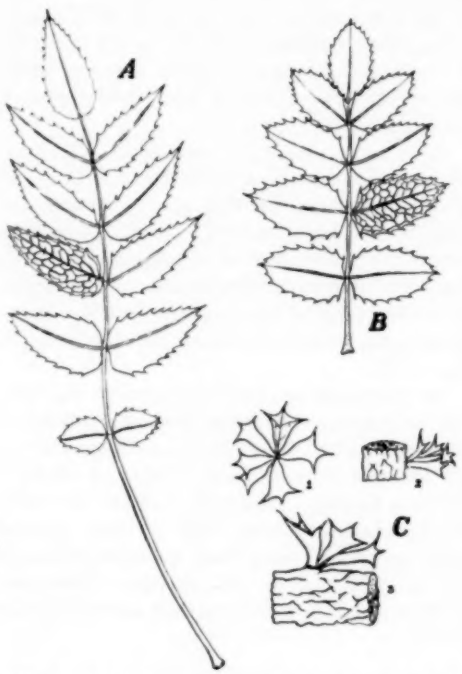


Fig. 2.—Sketch *A* represents a drawing of a leaf from *Berberis nervosa*. Sketch *B* represents a drawing of a leaf from *Berberis aquifolium*. Sketch *C* shows views of the leaves from *Berberis boliviana*.

Powdering.—All the types of berberis worked upon are extremely hard and difficult to reduce to a fine

powder. The difficulty can be overcome only by repeating the milling process several times with a good mill.

Description of the Various Types of Berberis.—The drug *Berberis aquifolium* consists of the dried roots and rhizomes and occurs on the market in a variety of forms. Entire pieces are usually 10–30 cm. in length and from 1 to 4.5 cm. in diameter. The drug may also be found segmented into pieces of various sizes. Usually only the thicker pieces are segmented, mostly the ones that are broader than 2 cm. in diameter. These thicker pieces, especially the rhizomes and less frequently the roots, whether segmented or entire, may be marked with a few radial splits which start from the periphery and work their way into the inner tissues, often approaching the pith, or the center. The segments are from 4 to 8 cm. in length, and vary in thickness. The roots as well as the rhizomes are cylindrical. The roots are more knotty than the rhizomes, and are densely and irregularly branched. Both rhizomes and roots are densely covered with a grayish-brown to greenish-brown periderm. The periderm of the root is of a darker shade than that of the rhizome, and the rhizome periderm is occasionally covered with a greenish moss or lichen growth.

The periderm of the rhizome, which is usually about 1–2 mm. thick, according to the entire diameter of the organ, is longitudinally wrinkled and interlaced with shorter transverse markings. The periderm of the root is more or less evenly covered with deep longitudinal fissures which are interlaced with shallower and smaller oblique markings that form a more uniform meshwork than on the rhizome.

The drug is extremely hard, heavy, tough, and compact in the inner woody layers, and comparatively weak and splintery in the periderm.

The wood is of a bright greenish-yellow color, and the intensity of the color varies greatly from piece to piece. The color is always more pronounced when the drug is moistened with water or alcohol. The wood shows distinct, narrow rings of growth, with very predominant narrow wood rays and a comparatively narrow area of pith, which is sometimes eccentric.

The fracture of the root or rhizome is tough, and not easily broken, even when it is less than a centimeter in diameter. When broken it exposes a fibrous, very uneven, splintery surface. Usually the periderm separates in layers before the woody tissues break apart.

The wood and bark have an intensely bitter taste when chewed, and color the saliva and the mucous membrane a vivid yellow. They are devoid of any characteristic odor.

The drug *Berberis nervosa* also consists of dried roots and rhizomes. The description of this drug is very similar to that of *Berberis aquifolium* except for some minor and not very constant differences noted when it is examined for gross structure.

The color of the wood is less yellow in intensity, especially in the pith region, and the pith occupies a proportionally larger area than in *Berberis aquifolium*.

The periderm of the rhizome and roots of berberis obtained from *Berberis nervosa* is more brownish silvery gray, and is lustrous. It is covered with deeper transverse markings than are found in *Berberis aquifolium*.

In all other organoleptic respects the characteristics of *Berberis nervosa* are similar to those of *Berberis aquifolium*.

Bolivian berberis is the root and rhizome of *Berberis boliviana*. Its morphological description is very much the same as that of *Berberis aquifolium*, except for some very small and negligible variations, such as size and color. Commercial Bolivian berberis is found in the segmented state, since it usually consists of fragments obtained from organs of rarely less than 3 cm. in diameter, the range usually being from 2 to 6 cm.

The periderm is usually more adherent and the surface slightly less wrinkled than that of *Berberis aquifolium*, or *Berberis nervosa*. The wood is tougher, a darker yellow in color, and without the greenish tinge.

Gross Structure of Berberis.—A study in the gross structure of the three types of berberis rhizomes shown in Fig. 3, sketches A, C, and E, disclose a general similarity in most of the tissues except the phloem region. While *Berberis nervosa* and *Berberis boliviana* rhizomes have lignified fibers in their phloem region, more in the *nervosa* than in the *boliviana*, *Berberis aquifolium* rhizome has no lignified fibers in this region.

The roots as seen in Fig. 3, sketches B, D, and F, are also very similar in most respects, except that *Berberis nervosa* root, like its rhizome, has numerous

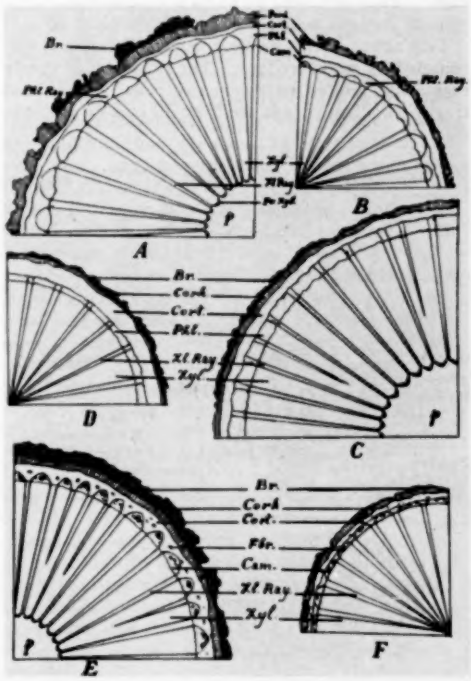


Fig. 3.—Sketches *A*, *C*, and *E* represent diagrams of cross sections of *Berberis* rhizomes. Sketches *B*, *D*, and *F* represent cross sections of *Berberis* roots. *Perd*, periderm; *Cort*, cortex; *Phl*, phloem; *Cam*, cambium; *Phl. Ray*, phloem ray; *Xyl*, xylem; *Xl. Ray*, xylem ray; *Pr. Xyl*, primary xylem; *p*, pith; *Br*, rhytidome; *Fbr*, fibers; *A*, *B*, *B. aquifolium*; *D*, *C*, *B. boliviana*; *E*, *F*, *B. nervosa*.

groups of lignified fibers in the phloem region, while the other two types of roots do not.

Histolology of Berberis.—A complete study of these three berberis rhizomes was made and their cross sections are here depicted with the help of camera lucida drawings (Fig. 4).

As fairly complete histological accounts of the rhizome of *Berberis aquifolium* are found in Youngken (9), the National Formulary VII (1), and Gathercoal and Wirth (12), the histological description will be limited to *Berberis nervosa* and *Berberis boliviana* rhizomes.

Berberis Nervosa Rhizome.—While a general similarity in the sequence of the common tissues exists in all of the three different *Berberis* rhizomes examined, the most outstanding characteristic of *Berberis nervosa* rhizome is the phloem region. This region has well-developed and lignified fibers, which are usually in groups (although occasionally isolated), and form compact strengthening layers in the phloem region.

The pith at the center occupies from $^1/_4$ to $^1/_3$ of the entire diameter of the rhizome, and is more often slightly eccentric. The pith cells at the outer region are thick-walled and heavily lignified, with many irregularly arranged simple pits. In the center of the pith region the cells are thin-walled, and slightly lignified. The pith cells of *Berberis nervosa* are further characterized by being slightly heterogeneous with some very rigid cells and many more less rigid ones. They are from 25 to 85 μ in diameter. Pith cells of *Berberis aquifolium* vary from 13 to 50 μ.

Pith tissue in the *Berberis nervosa* rhizome is surrounded by wood wedges. The cells in this woody region are limited to xylem ray cells, vessels, and tracheids. The vessels are from 10 to 40 μ in diameter, and from 165 to 231 μ in length. The vessels of *Berberis aquifolium* are usually 10 to 27 μ in diameter, and from 125 to 165 μ in length. The shape of the vessels is very similar to those found in other species of *Berberis*, as seen in Fig. 5, sketches 1 to 11. The vessels in *Berberis nervosa* rhizome are arranged in groups of twos, less frequently in threes, and their walls have bordered pits arranged spirally.

The tracheids are lignified, thin-walled, with broad lumen, pointed ends, and simple rounded pits which are arranged irregularly as shown in Fig. 5, sketches *d*, *e*, and *f*.

The cambium layer consists of collapsed cells, and the phloem consists of elements which make it easy to distinguish this species of *Berberis* from the other two. The phloem ray bands, which begin at the cambium line, gradually become broader and finally merge at the cortex, leaving above each xylem band a half-moon shaped area of phloem tissue which is almost always occupied by one or more dense groups of strongly lignified fibers. When these fibers are in one single group in a phloem patch, they usually form a triangular structure, which occupies most of the semi-lunar area; but when these lignified elements are in more than one patch, they may be observed in chains or in isolated form. When in chains they are tangentially placed. Lignified fibers are not limited to this area; they also occur irregularly in the cortex, where they are usually isolated, and may be considered to be cortico-pericyclic fibers.

The lignified fibers of the phloem region are longer than and different from the xylem tracheids, being branched at their ends, and the thickening on their walls is in the shape of simple circular openings and oblique slits, all arranged in spiral fashion as seen in Fig. 5, sketches *a*, *b*, and *c*.

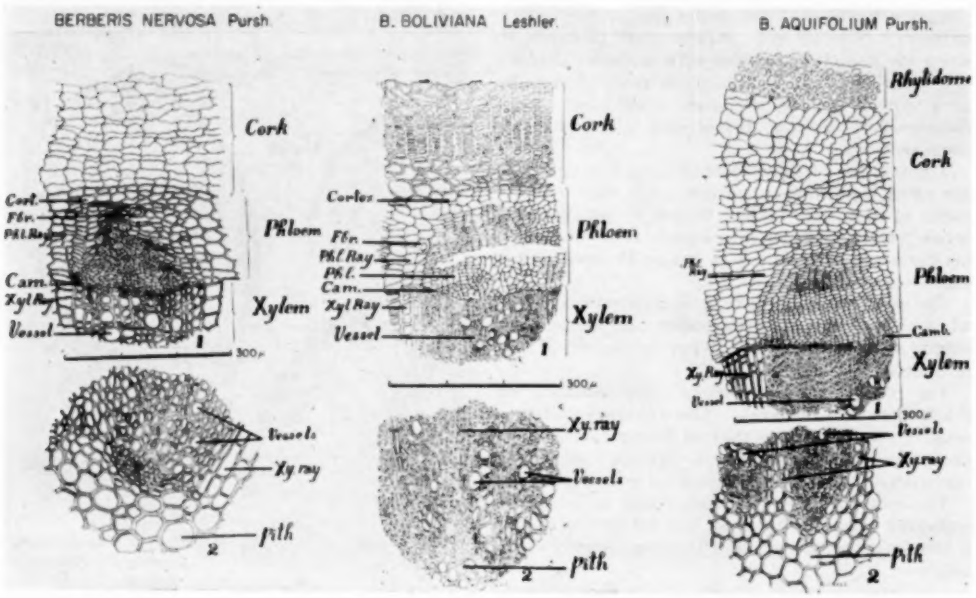


Fig. 4.—Detailed camera lucida drawings of representative segments of cross sections from *Berberis nervosa*, *Berberis boliviana*, and *Berberis aquifolium*, showing most of the phloem and the xylem tissues nearest to the cambium line; together with tissues at a region which includes pith and portions of the xylem. *Cort*, cortex; *Fbr*, fiber; *Phl. Ray*, phloem ray; *Cam*, cambium; *Xyl. Ray*, Xylem ray.

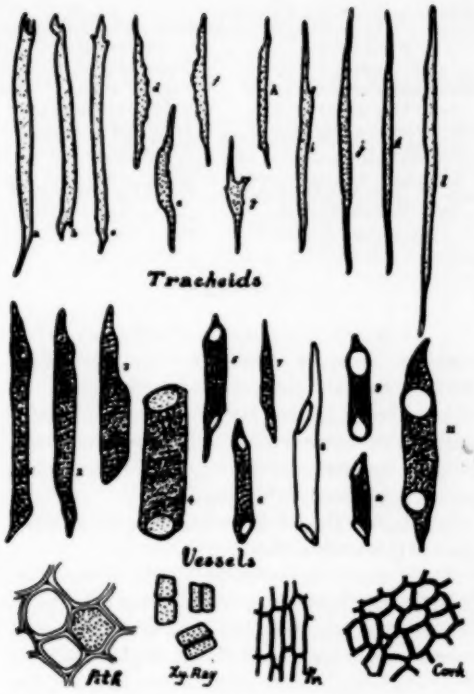


Fig. 5.—Drawings of tracheids, vessels, and pith cells. *a*, *b*, and *c* are tracheids from the phloem of *Berberis nervosa*. *d* and *e* are tracheids from the xylem of *Berberis nervosa*. *f*, *g*, *h*, and *i* are tracheids from *Berberis boliviana*. *j*, *k*, and *l* are tracheids from *Berberis aquifolium*. *1*, *2*, *3*, and *4* are vessels from *Berberis nervosa*. *5*, *6*, and *7* are vessels from *Berberis boliviana*. *8*, *9*, *10*, and *11* are vessels from *Berberis aquifolium*.

Starch grains are found in all parenchymatous cells. They are most abundant in the ray cells as single, large, spherical grains with a distinct hilum in the form of a point in the center of each grain. They are devoid of any striations. The largest grain measured 17 μ, while most of the grains varied from 5 to 13 μ. No calcium oxalate crystals were observed.

Berberis Boliviana Rhizome.—As seen in Fig. 3, sketch C, the pith occupies $^1/_2$ of the diameter of the rhizome, and the pith cells are more or less uniform in their lignification. They possess simple pits as seen in Fig. 5 (pith). The pith cells vary in size from 16 to 56 μ in diameter, while the pith cells of *Berberis aquifolium* measured 13–50 μ.

The xylem tissue which occupies the bulk of the remaining area consists of xylem strands which are about 250 μ at their edge nearest the cambium line and which extend inward, gradually tapering into a wedge of from 70 to 100 μ in width. The tips of these xylem bands consist of a condensed mass of xylem elements representing the primary xylem.

The vessels occur in large groups in the primary xylem wedge area, but further out they become evenly diffused and porous; their size varies from 10 to 35 μ in diameter, and their length varies from 165 to 260 μ. These vessels have bordered pits, elongated pointed ends, and elliptical to circular openings in the side of the walls nearest the two ends, as seen in Fig. 5, sketches 2, 5, 6, and 7.

The tracheids are fairly uniform in diameter, being from 10 to 14 μ wide, and from 324 to 480 μ long, with polygonal outline in cross section. Their walls have simple pits and oblique slits which are arranged spirally or irregularly.

The xylem ray cells are rectangular in outline, arranged in bands of 2 to 7 cells situated distinctly in radial direction, and ending in the pith region. Usually, the ends are 2 cells in thickness. Xylem ray cells have thick lignified walls with simple pits, and usually contain large, single, but sometimes two smaller, prismatic calcium oxalate crystals. The crystals are rhombic in shape and vary in size from 13 to 33 μ. There is also starch in these cells. The starch grains found in *Berberis boliviana* rhizome are usually simple, but some are compound grains of twos and threes. The starch grains vary in size from 7 to 24 μ, and the commonest size is 16.5 μ.

The phloem parenchyma is radially divided by the phloem rays to form patches coincident with the xylem patches. The phloem ray cells are rectangular to rounded, with cellulosic cell walls. Each phloem ray is about 4 cells wide, increasing gradually in width as it proceeds toward the cortex with which it merges to form a continuous layer, leaving the phloem parenchyma in almost half-moon shaped compact masses above each xylem band.

Phloem ray cells, and less frequently other parenchyma cells of the cortex, contain well-developed, large, rhombic crystals of calcium oxalate, similar to the ones found in xylem ray cells.

Often in the phloem ray region, and sometimes in the entire phloem region, there are seen a few isolated, well-developed fibers with lignified walls, polygonal in outline when seen in cross section, and with a fairly large lumen. These fibers are usually 25 μ in diameter, and about 190 μ in length.

Comparative Study of the Elements Found in the Three Types of Berberis.—The histological study of these closely related rhizomes having been made and the variations detected in cell structures and cell dimensions outlined, these measurements and the cell contents have been grouped in Table I which will serve as a ready reference whenever the establishment of the botanical origin of a commercial form of *Berberis* is required.

Periderm.—It is difficult to attach much importance to the characteristics of cells from the periderm region which usually consist of lignified and suberized cork cells.

Phloem and Pericyclic Fibers.—Lignified phloem fibers are limited to *Berberis nervosa* and *Berberis boliviana* rhizomes. They are absent in the rhizome of *Berberis aquifolium*. Fibers are more abundant in *Berberis nervosa* than in *Berberis boliviana*. They are usually branched at both ends, as seen in Fig. 5, sketches *a*, *b*, and *c*.

Tracheids.—Tracheids in all three types of berberis are heavily lignified; the widest are found in *B. nervosa* and they vary in size from 13 to 20 μ. Their lengths vary from 324 to 540 μ. The average length of the 100 measured was 420 μ. The tracheids in *boliviana* are the narrowest of the three species, varying from about 10 to 14 μ in diameter. The average width of the 100 measured was 12 μ, and their lengths varied from about 324 to 486 μ. The average length of the 100 measured was 378 μ.

TABLE I.—SUMMARY OF CELL DIMENSIONS AND CELL CONTENTS[a]

Elements	*B. boliviana*	*B. aquifolium*	*B. nervosa*
Vessels	10 to 35 μ wide; 165 to 260 μ long	10 to 27 μ wide; 125 to 165 μ long	10 to 40 μ wide; 165 to 231 μ long
Tracheids	10–12–14 μ wide; 324–378–486 μ long	9–12–15 μ wide; 288–350–468 μ long	13–15–20 μ wide; 324–420–540 μ long
Pith cells	16 to 56 μ	13 to 50 μ	25 to 85 μ
Calcium oxalate crystals	13 to 33 μ	None	None
Starch grains	7–16.5–24 μ, simple or in twos or threes	3–10–20 μ simple and in twos, threes, or fours	6–9–13 μ simple only

[a] The underlined figures represent the most common sizes.

B. aquifolium containes tracheids which vary in diameter from 9 to 15 μ. The average of the 100 measured was 12 μ. The lengths varied from 288 to 468 μ, the average of 100 measured being 350 μ.

Vessels.—There is a general similarity among the vessels found in all three types of berberis, all being lignified and mostly all with pointed ends. They bear bordered pits on their surfaces with their end openings usually on the side walls nearest the pointed ends.

The vessels in *B. nervosa* approach a typical advanced shape, their end openings being large, circular, and on their transverse walls. These vessels are the broadest in diameter, varying from 10 to 40 μ wide, with lengths varying from 165 to 231 μ.

Vessels from *B. boliviana* range from 10 to 35 μ in diameter, and from 165 to 260 μ in length.

B. aquifolium vessels are the narrowest and shortest, being from 10 to 27 μ in diameter, and from 125 to 165 μ in length.

Pith Cells.—The largest pith cells are found in *nervosa*, being from 25 to 85 μ in diameter. Pith cells from *boliviana* varied from 16 to 56 μ. Those of *aquifolium* are the smallest, measuring from 13 to 50 μ.

CELL CONTENTS

Calcium Oxalate.—Calcium oxalate crystals are found only in the *boliviana* drug, and are rhombohedral in shape. These crystals are usually found in the ray cells, often one large crystal or occasionally two smaller crystals in a cell.

No calcium oxalate crystals were seen in the other two types of berberis.

Starch.—Starch occurs in the ray cells and cells of the phloem parenchyma, and in some of the pith cells. The *nervosa* drug contains simple, spherical grains uniform in size, varying from 6 to 9 μ in diameter. Starch in *boliviana* occurs in compound and in simple form, each grain being spherical and the sizes varying from about 7 to 26 μ. *Aquifolium* also contains compound and simple grains of starch, their sizes varying from 3 to 20 μ.

SUMMARY

1. A report is given of a study of American berberis obtained from *Berberis aquifolium*, *Berberis nervosa*, and *Berberis boliviana*.

2. A comparative study of the overground parts of the three species of *Berberis* is reported. A plate representing photographs of authentic herbarium specimens is included.

3. An outline of the material and methods used in this study is given.

4. A comparative study of the gross structures of the rhizomes and roots of the three types of berberis is reported, and illustrations depicting their outlines are included in a single plate.

5. Detailed histological descriptions of the rhizome of *Berberis nervosa* and of *Berberis boliviana* are here reported for the first time, and a plate containing three camera lucida drawings of the three *Berberis* rhizomes is shown.

6. A comparative study of the tissue elements such as would be found in the powdered drug of all three types of *Berberis* is reported.

REFERENCES

(1) "The National Formulary," 7th ed., Mack Publishing Co., Easton, Pa., 1942.
(2) Lechler, W., "Berberides Americae Australes Descripsit," Stuttgartiae Sumtibus Librarae Schweizerbart, 1857.
(3) Brahmachari, U., *Indian Med. Gaz.*, **79,** 259(1944).
(4) Das Gupta, B. M., and Dikshit, B. B., *ibid.*, **64,** 67 (1925).
(5) Jolly, G. G., *ibid.*, **46,** 466(1911).
(6) Varma, R. L., *ibid.*, **62,** 84(1927).
(7) Karamchandani, P. V., *ibid.*, **62,** 558(1927).
(8) "The Dispensatory of the United States of America," 24th ed., J. B. Lippincott Co., Philadelphia, 1947.
(9) Youngken, H. W., "Textbook of Pharmacognosy," 6th ed., The Blakiston Co., Philadelphia, 1948.
(10) "The British Pharmaceutical Codex," The Council of the Pharmaceutical Society of Great Britain, The Pharmaceutical Press, London, 1934.
(11) Short, G. R. A., *Chemist and Druggist*, **105,** 262 (1926).
(12) Gathercoal, E. N., and Wirth, E. H., "Pharmacognosy," 2nd ed., Lea & Febiger, Philadelphia, 1949.

Examination of the Seed of *Pachyrrhizus erosus*[*,†]

By BELLA Y. BAKER and E. V. LYNN[‡]

The seed of *Pachyrrhizus erosus*, commonly known as the yam bean, was examined several years ago as a possible commercial source of rotenone. Although the rotenone content is too small to make the seed of economic importance, several other poisonous substances are present, including pachyrrhizid, pachyrrhizone, erosone, and at least two saponins. The fatty oil, which constitutes about 20 per cent of the seed, is high in saturated glycerides and is nondrying. The unsaponifiable portion, about 1 per cent of the oil, contains saturated hydrocarbons and several phytosterols.

THE SEED of *Pachyrrhizus erosus* (L.) Urban (*P. angulatus* Rich.), commonly known as the yam bean, has been used by the natives of the East Indies and Brazil as a source of fish poison, and more recently has been examined for the amount of rotenone, which is small.

The seeds were first investigated in 1880 by Peckolt (1), who determined the amount of oil and starch. In 1890 Greshoff (2) isolated a poisonous, amorphous substance that he named pachyrrhizid. Van Sillevoldt (3) in 1899 separated pachyrrhizid in a purer state, two crystalline compounds melting at 106° and 207°, and a fourth substance that did not melt below 240°. Altamirano, *et al.* (4), in 1907 determined the amounts of glucose, resin, fat, mineral salts, and tannic acid. In 1933 Padilla and Soliven (5) analyzed the seeds for moisture, fat, protein, carbohydrate, and ash. Two years later Bouillenne, Bouillenne, and Koolhaas (6) isolated an amorphous compound, pachyrrhizine, m. p. 85–90°. Nag, *et al.* (7), in 1935—1936 made a proximate analysis, examined the oil, and showed that the aqueous extract is poisonous. In 1941 Hwang (8) reported that the seeds contain 0.56 to 1.06 per cent of rotenone, although he did not isolate the compound, but Hansberry and Lee (9) in 1943 found the percentage to be very low, as did also Norton (10) who identified the rotenone. Norton and Hansberry (11) in 1945 found also a compound that they called erosone. In 1946 Meyer (12) obtained from the ether extract a ketone pachyrrhizone melting at 248°.

The writers were chiefly concerned with studying the fatty oil and isolating the saponins that preliminary experiments indicated to be present in the seed.

* Received August 22, 1952, from the Massachusetts College of Pharmacy, Boston.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.
† Abstracted from a thesis submitted to the Graduate Council of the Massachusetts College of Pharmacy by Bella Y. Baker in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.
‡ Research Professor of Chemistry, Massachusetts College of Pharmacy.

EXPERIMENTAL

Proximate Analysis.—Weighed samples of the ground seeds were submitted to analysis according to methods of the U. S. P. and "Official Methods of Analysis of the Association of Official Agricultural Chemists." Successive extractions, each for twenty hours, were also made in a Soxhlet apparatus on duplicate samples. The figures for the results each represent the average percentage of two determinations: volatile oil, trace only; loss at 100°, 4.97; ash, 4.15; acid-insoluble ash, 0.21; crude fiber, 6.00; starch by diastase, 22.90; nitrogen x 6.25, 25.16; petroleum benzin extract, 23.46; diluted alcohol extract, 8.65; aqueous extract, 14.42; successive extractions: benzin 23.46, ether 1.09, alcohol 4.69, water 12.61.

Fatty Oil.—The extract by benzin, which was presumed to be chiefly oil, had the following characteristics: d^{25}, 0.9205; n^{25}, 1.4669; saponification value, 194.16. The oil was also prepared from 2.5 Kg. of dried seeds by extraction with ether. It was dark green, had a characteristic odor, and showed little tendency to dry. It possessed the following characteristics: d^{25}, 0.9425; n^{25}, 1.4700; saponification value, 170.89; unsaponifiable residue, 1.46%; acid value, 2.3; and iodine value, 123.00.

A quantity of the oil (147 Gm.) was saponified with alcoholic sodium hydroxide by boiling under a reflux condenser. The residue, after addition of water, was concentrated somewhat to remove the alcohol and then extracted with ether to separate the unsaponifiable portion. The aqueous solution was acidified with sulfuric acid to precipitate the insoluble fatty acids, which were removed by chilling and decantation. The soluble volatile acids, determined by distillation, were negligible. The soluble nonvolatile acids were neutralized with sodium hydroxide, and the resulting liquid was concentrated to a small volume, acidified, and extracted with ether. After drying, the ethereal solution was evaporated to dryness but yielded only a minute quantity of residue. A part of the insoluble acids was subjected to the Lane method (13) for the separation of solid and liquid acids by solubility of the lead salts in ether. The amount of solid acids was 46.05%; that of the liquid acids, 53.95%. Linoleic acid was identified by conversion to the tetrabromide melting at 113–114°, but is probably present only in small amounts. The principal liquid acid is oleic, which was identified by conversion to dihydroxystearic acid melting at 134–135°. By fractional crystallization the solid acids were separated into the following fractions: palmitic acid, m. p. 59–60°,

and its *p*-bromophenacyl ester, m. p. 85°; myristic acid, m. p. 49–50°, and its *p*-bromophenacyl ester, m. p. 79°; and a small amount of another acid, possibly lauric.

Unsaponifiable Residue.—The dried ether solution containing the portion that did not saponify was evaporated to dryness, leaving 5.98 Gm. or 4.07% of a brownish, gummy mixture. By treatment with hot alcohol and repeated recrystallization, about 0.5 Gm. of a wax-like solid and less than 10 mg. of needles were obtained. The former, which melted at 62–65° and gave no test for unsaturation, is probably a saturated hydrocarbon. The colorless crystalline material melted at 127–128° and gave a positive Liebermann-Burchard test for sterol. The main portion of the unsaponifiable residue appeared to consist of resin mixed with the compounds obtained in the alcoholic extract.

Alcoholic Extract.—A quantity (1.5 Kg.) of coarsely ground seeds was extracted with petroleum benzin to remove the fat. The dried residue was exhausted with alcohol by percolation, and the alcohol was partially distilled off at about 15 mm. pressure. The residual brown liquid, upon cooling, deposited an amorphous mass, from which a small portion that melted at about 80° was extracted with ether. This substance corresponded with Greshoff's pachyrrhizid, but it could be separated into several amorphous substances that melted indefinitely at various temperatures up to 123°. There was also obtained a very small amount of a colorless crystalline compound that melted at 154–155° and was probably rotenone. The deposit from the alcoholic extract and the extract itself also gave an abundant frothing with water and probably contained saponins. From that portion of the alcoholic extract that was not dissolved by ether, a yellowish-brown mass was removed by chloroform; from this was separated a minute quantity of colorless crystals melting at 265–268°, and by digestion with acetic acid and acetone about 0.9 Gm. of yellow crystals melting at about 200° was obtained. Repeated recrystallization of the latter from benzene lightened the color somewhat and raised the melting point to 239°. This compound corresponds with Meyer's pachyrrhizone.

Aqueous Extract.—Attempts to percolate the ground seeds with water met with failure because the solution was highly viscous and did not penetrate through the seeds. A clear, mobile extract was finally obtained from 1.5 Kg. of the defatted seeds by maceration, straining through muslin, and heating the turbid, viscous liquid to coagulate albumins. The dried coagulum amounted to 2.1% of the seeds and contained nitrogen. The clear, mobile filtrate foamed considerably on shaking and laked blood even in great dilution.

Addition of lead acetate to the solution gave a copious white precipitate which was filtered and then freed from lead by means of hydrogen sulfide. The filtrate from lead sulfide laked blood and upon evaporation left a brownish residue calculated as 0.2% of the seeds. It redissolved in water and gave only a slight reduction of Fehling's solution. Because attempts to crystallize or purify the filtrate failed, it was hydrolyzed by boiling with concentrated hydrochloric acid for two hours. The neutralized residue, which strongly reduced Fehling's solution, after some concentration and cooling yielded a small amount of amorphous yellowish deposit that could not be crystallized. From the residual liquid, a similar amorphous substance that melted at 75–77° was extracted with ether.

The filtrate from lead acetate gave a yellowish precipitate with lead subacetate, which was freed from lead as before. The resulting solution, which laked blood and slightly reduced Fehling's solution, was evaporated to yield about 0.7% of light brown residue. After hydrolysis this residue strongly reduced Fehling's solution, but all attempts to crystallize it or its hydrolytic products failed. Further study with larger amounts of seeds is contemplated.

SUMMARY

1. The fatty oil was isolated and partially characterized, and a proximate analysis of the seeds was made.

2. Besides the substances previously found, at least two saponins are shown to be present.

REFERENCES

(1) Peckolt, T., *Botan. Jahresber.*, **8**, 451(1880).
(2) Greshoff, M., *Ber.*, **23**, 3539(1890).
(3) van Sillevoldt, H. E. T., *Arch. Pharm.*, **237**, 595 (1899).
(4) Altamirano, F., Ramirez, J., Villasenor, F. F., Armadaris, E., and Martinez del Campo, J., *Inst. Med. Nac.*, **4**, 1(1907).
(5) Padilla, S. P., and Soliven, F. A., *Philippine Agr.*, **22**, 408(1933).
(6) Bouillenne, R., Bouillenne, M., and Koolhaas, D. R., *Congr. intern. tech. et chim. ind. agr., Compt.-rend. 4e Congr. Bruxelles*, **2**, 333(1935).
(7) Nag, N. C., Banerjee, H. N., and Pain, A. K., *Trans. Bose Research Inst. Calcutta*, **11**, 83 (1935–1936).
(8) Hwang, S. L., *Kwangsi Agr.*, **24**, 28(1941).
(9) Hansberry, R., and Lee, C., *J. Econ. Entomol.*, **36**, 351 (1943).
(10) Norton, L. B., *J. Am. Chem. Soc.*, **65**, 2259(1943).
(11) Norton, L. B., and Hansberry, R., *ibid.*, **67**, 1609 (1945).
(12) Meyer, T. M., *Rec. trav. chim.*, **65**, 835(1946).
(13) Lane, N. J., *J. Am. Chem. Soc.*, **15**, 110(1893).

Note

A Note on the Isolation of Furan-3-Carbonic Acid from the Seeds of *Euonymus europaea* L.*

By EGIL RAMSTAD

THE fruits of *Euonymus europaea* L. have enjoyed a variety of uses in folk medicine in the past (1). Doebel and Reichstein (2) succeeded in isolating 0.1 per cent of three crystalline alkaloids, and Santavy and Reichstein (3) obtained two cardiac glycosides, euonoside A and B, from the seeds of this plant. The use of this drug as an insecticide was tested recently and found to be reliable (4).

During the present investigation of the chemical basis for the insecticidal activity of the seeds, an organic acid was isolated and identified as furan-3-carbonic acid.

EXPERIMENTAL

Extraction.—A quantity of 1 Kg. of seeds of *Euonymus europaea* L., collected on the Purdue University campus was finely powdered and extracted in a Soxhlet apparatus for forty-eight hours with petroleum ether (b. p. 30–60°). Yield: 465 Gm. of a red-orange oil of slightly bitter taste.

Isolation.—A quantity of 400 Gm. of the oil was shaken three times with 200 ml. of 95% alcohol. The combined alcoholic extracts were concentrated *in vacuo* to about 50 ml., then refluxed for one hour with 50 ml. of a 5% alcoholic potassium hydroxide solution. The solution turned dark brown and developed a strong aromatic odor. It was diluted with 200 ml. of water, and the unsaponifiable portion, including the odoriferous substance, was shaken out with pentane. The aqueous layer was acidified with sulfuric acid and extracted three times with 100 ml. of chloroform. The dark brown semi-solid residue that remained after distillation deposited heavy crystals upon standing.

The crystal mass was washed by stirring with petroleum ether, in which solvent the crystals were insoluble. They were dissolved in ether, however. The ether residue was sublimed at 100° (5) to form white, shiny platelets. Volatile fatty acids which were present in the sublimate were separated by distillation *in vacuo*.

Properties.—The compound has a melting point of 122–123° (uncorr.) [reported (6), 120°; (5) 121–122°]. It is insoluble in petroleum ether, sparingly soluble in water, and soluble in ether, chloroform, and alcohol. It liberates carbon dioxide from an aqueous solution of sodium bicarbonate. No acetylation product could be obtained with acetic anhydride in pyridine. It possesses no optical activity. When it was treated with a chromic acid mixture, no odor of benzaldehyde resulted (cinnamic acid). It gave no reaction with ferric chloride.

A mixed melting point with benzoic acid (m. p. 123–124°) resulted in a depression to 85–95°.

Analysis.—The Lassaigne test for nitrogen was negative.

Titration with alkali showed a molecular weight of 110 (0.0353 Gm., 6.45 ml. of 0.05 *N* NaOH), indicating a monobasic acid.

The molecular weight, determined according to Rast's method (7) with camphor (m. p. 178°), was found to be 114.8 and 111.1 (theoretical, 112).

Anal.—Calcd. for $C_5H_4O_3$: C, 53.6; H, 3.6. Found: C, 53.5; H, 3.7.

These data could also indicate the isomer furan-2-carbonic acid, which, however, has a melting point of 133–134° (8) and sublimes in the form of needles.

The fact that the acid could not be extracted from the lipid fraction with alkali, but was obtained from hydrolysis of an alcoholic extract of the fat, indicates that the acid occurs in the seed in the form of an ester.

SUMMARY

An acid has been isolated from seeds of *Euonymus europaea* L. The properties, constants, elementary composition, and molecular weight of the acid show it to be identical with furan-3-carbonic acid.

REFERENCES

(1) Dragendorff, G., "Die Heilpflanzen der verschiedenen Volker und Zeiten," Stuttgart, 1898, p. 400.
(2) Doebel, K., and Reichstein, T., *Helv. Chim. Acta*, **32**, 592(1949).
(3) Santavy, P., and Reichstein, T., *ibid.*, **31**, 1655(1948).
(4) Tattersfield, F., *et al.*, *Roy. Botanic Gardens, Kew, Bull. Misc. Inform.*, **3**, 329(1948).
(5) Rogerson, H., *J. Chem. Soc.*, **101**, 1040(1912).
(6) Power, F., and Salway, A., *Pharm. J.*, **90**, 550(1913).
(7) Rast, K., *Ber.*, **55B**, 1051, 3727(1922).
(8) Hill, I. A., *Am. Chem. J.*, **3**, 38(1881).

* Received August 22, 1952, from the School of Pharmacy, Purdue University, Lafayette, Ind.
Presented to the Scientific Section, A. PH. A., Philadelphia meeting, August, 1952.

Book Notices

An Introduction to the Chemistry of the Hydrides. By DALLAS T. HURD. John Wiley and Sons, Inc., New York, 1952. x + 231 pp. 23.5 x 15.5 cm. Price $5.50.

The aim of this book is to present, in a critical and coordinated manner, the theory, properties, and reactions of the hydrides of industrial and academic interest to chemists.

The introduction orients the reader to the chapters that follow. The chemical bonding and structure of the hydrides and general considerations involving the ionic hydrides are adequately discussed. Other chapters cover the hydrides of the alkali and alkaline earth metals, and the hydrides of beryllium and magnesium. The covalent hydrides are discussed in a general way and chapters are included on the hydrides of elements belonging in groups III to VII inclusive. Other subjects discussed are the complex hydrides and those of the transitional borderline elements such as copper, cadmium, and thallium. The nomenclature of these compounds is also thoroughly reviewed in a separate chapter.

The book is provided with five appendices, an author index, and a subject index, and is well documented throughout. The compilation of this data in one volume is most useful, particularly in regard to the time saved which would otherwise have to be devoted to a search of the literature.

Pharmacology in Clinical Practice. By HARRY BECKMAN. W. B. Saunders Co., Philadelphia, 1952. xx + 839 pp. 17 x 25 cm. Illustrated. Price $12.50.

The author, who is Director of the Department of Pharmacology at Marquette University Schools of Medicine and Dentistry and Consulting Physician to the Milwaukee County General Hospital and Columbia Hospital in Milwaukee, has written this book as a successor to his *Treatment in General Practice.* The volume is divided into two sections: the first presents the pharmacologic aspects of major problems with which the physician and dentist are frequently confronted; the second is devoted to a compendium in which certain fundamental chemical and physical facts about the drugs discussed in Section I are made available. As the title suggests, the book is written from the clinical standpoint. It presents information in terms of specific diseases and their symptoms rather than in terms of anatomical groupings of organs or chemical groups of drugs, and is designed to orient the physician and the dentist at the graduate level to the applications of pharmacology in the practice of their professions.

While designed specifically for physicians and dentists, this volume could also be used by the professional pharmacist as a source of information when he is consulted by physicians on the choice of drugs for specific conditions.

Although the information is presented in an authoritative manner, selected references to original literature are presented at the end of each chapter. The book is also provided with an unusually complete subject index, so that information on either specific drugs or the conditions they are designed to treat may be easily located.

Blakiston's Illustrated Pocket Medical Dictionary. NORMAND L. HOERR and ARTHUR OSOL, Editors. The Blakiston Co., New York, 1952. Illustrated. xvi + 1005 pp. + 24 pp. of illustrations. 12.5 x 18 cm. Price $3.75.

This compact pocket dictionary has been designed to meet the needs of pharmacists, laboratory workers, nurses, and dentists. It contains over 33,000 entries with complete definitions, and includes a number of useful reference tables unusual in books of this type. In addition, a number of illustrations not ordinarily found in a pocket dictionary have been placed at the end of the book for easy reference.

The arrangement of material appears to follow a logical and practical pattern. Terms are entered primarily under the noun form with adjectival and adverbial derivatives following. Pronounciation is shown by syllable division and accent, or by phonetic spelling where necessary to eliminate the use of complicated symbols. Many new medical terms are drawn from textbooks and medical journals appearing during the past three years and are included for the first time in any medical dictionary.

The book is characterized throughout by the inclusion of a larger number of chemical and pharmaceutical terms than expected in a medical dictionary, and can be recommended for anyone dealing with medical, chemical, or pharmaceutical terms.

Maleic Anhydride Derivatives. Reactions of the Double Bond. By LAWRENCE H. FLETT and WILLIAM HOWLETT GARDNER. John Wiley and Sons, Inc., New York; Chapman and Hall, Ltd., London, 1952. x + 269 pp. 15.5 x 23.5 cm. Price $6.50.

This book, designed for students and research workers interested in synthetic organic chemistry who are confronted with the necessity of finding suitable chemicals to solve specific problems, consists of a presentation of more than 100 different types of chemical reactions involving maleic anhydride or one of its simple unsaturated derivatives.

The material on each compound discussed is restricted to a set of two facing pages. On the left-hand page is given a description of the reaction products and possible uses of the chemical under discussion; the right-hand page includes an equation for the reaction involved, and a brief description of a method of preparation. The method described, however, is not necessarily intended to be followed as a laboratory procedure, and anyone undertaking the preparation of any of the compounds should first consult the references cited. Many of the compounds and reactions discussed are of special

pharmaceutical interest, e. g., succinic acid, pyrazinotetrahydrophthalic anhydride, pyrazolinecarboxylic acids, hydroxysuccinic acids, and many others.

The book is divided into eight chapters covering the hydrocarbons, halogens and their compounds, hydrogen, metallic compounds, compounds containing nitrogen, compounds containing oxygen, sulfur compounds, and energy and catalysts. The index, which is excellent, lists not only the compounds and the reactions involved in their preparation, but also their applications in various industrial fields.

Encyclopedia of Chemical Technology. Vol. IX. RAYMOND E. KIRK and DONALD F. OTHMER, Editors. Interscience Publishers, Inc., New York, 1952. xvi + 943 cm. 19.5 x 26.5 cm. Illustrated. Price $30.00 (Single Copy); $25.00 (Subscription).

The first volume of the *Encyclopedia of Chemical Technology* was reviewed [THIS JOURNAL **37**, 130 (1948)], and its design and plans for subsequent volumes were described in detail. The ninth volume in the series has now been published and covers subjects from Metal Surface Treatment through Penicillin.

Many articles of special pharmaceutical application are included in this volume. Of particular interest is the succinct treatment of nicotinic acid and nicotinamide by the late James F. Couch of the Eastern Regional Research Laboratories in a nine-page article. The physical and chemical properties, analysis, occurrence, preparation and manufacture, biological properties and uses, toxicity, and derivatives are adequately discussed. This treatment is representative of other sections relating to drugs and drug products. Also included are chapters on essential oils and penicillin.

Cross-reference indexing to items treated generically in previous volumes is used frequently throughout the text.

When completed, the *Encyclopedia of Chemical Technology* will include more than 1,000 authoritative articles, in alphabetical arrangement, by experts from the American chemical industry and from research institutions, and will constitute an exceptionally useful reference in any scientific library to serve as a starting point for the development of information on many subjects of general and specific interest.

Chemicals of Commerce. 2nd ed. By FOSTER DEE SNELL and CORNELIA T. SNELL. D. Van Nostrand and Co., Inc., New York, 1952. viii + 587 pp. 22.5 x 14.5 cm. Price $6.50.

The second edition of this useful reference volume has been thoroughly revised. As in the first edition, many pertinent facts about a large number of commercially available chemicals employed in the chemical industry are presented in concise form. It is written as simply and nontechnically as possible and summarizes a wealth of otherwise unobtainable information.

The book is divided into thirty-eight chapters, each covering a separate class of compounds beginning with the inorganic acids and ending with the vitamins, sterols, antibiotics, and hormones. The compounds discussed in each chapter are arranged in more or less alphabetical order, but the value of the book would have been greatly enhanced if it had been provided with a subject index. This omission is unfortunate because the nontechnically trained individual will undoubtedly find it difficult to locate certain compounds, due to lack of knowledge of their chemical classification.

The book is produced by an offset method, and the reproduction and binding are of excellent quality.

The Literature on Streptomycin, 1944–1952. By SELMAN A. WAKSMAN. Rutgers University Press, New Brunswick, N. J., 1952. xii + 553 pp. 16 x 23.5 cm. Price $5.00.

The isolation of streptomycin was announced in January, 1944. By 1948, approximately 1,200 articles dealing with this antibiotic had been published, and in order to make a bibliography of these articles available for convenient use, Dr. Waksman compiled the first edition of *The Literature on Streptomycin.*

In the interim between the appearance of the first edition and the recent publication of the second edition of this book, the number of articles dealing with streptomycin has increased to nearly 6,000. The second volume of literature references has been compiled in order to make available to the medical profession recent information on the several uses of streptomycin either alone or in combination with other drugs for the treatment of infectious diseases of man and of animals, for animal feeding, and for the preservation of semen and viruses.

The listing of the references and the arrangement of the book in general follow the style of the first edition. The subject index has been completely revised and is much more detailed in scope than that which appeared in the first edition. As further literature on streptomycin accumulates, annual supplements to the second edition are planned.

The author has performed a useful service in making available so complete a bibliography on such an important antibiotic as streptomycin.

Yearbook of the French Chamber of Commerce of the United States, Inc.—1952. 630 Fifth Avenue, New York, N. Y. 392 pp. 16 x 24 cm.

The French Chamber of Commerce of the United States was founded in 1896 and since that time has offered many services and facilities in the establishment of suitable contacts between producers, manufacturers, exporters and importers, and distributors and dealers in the United States and in France.

The *1952 Year Book* includes a wealth of information not only of commercial use, but also of value to tourists planning to visit France. One feature of especial interest is a section in reference to French patents, trade marks, trade names, and designs.

Introductory Mycology. By CONSTANTINE JOHN ALEXOPOULOS. John Wiley and Sons, Inc., New York, 1952. Illustrated. xiii + 482 pp. 23.5 x 15.5 cm. Price $7.00.

Until relatively recently, mycology constituted a highly specialized branch of science. With the discovery and development of antibiotics during recent years, a greater interest in this field has arisen. In fact, a general knowledge of mycology is be-

coming a part of the cultural background of every well-informed individual.

This book has been designed especially for the student or general reader who knows little or nothing about the fungi, but who is interested in obtaining an orderly presentation of certain fundamental concepts of the structure and classification of these organisms, without delving into the innumerable details and exceptions considered by the specialist.

The author has discussed the structure of the fungi and the life histories of various representative organisms. In his effort to answer the question, "What are fungi and how do they affect us?" he has succeeded admirably, and his work should find acceptance by students and general readers who are neither specialists in bacteriology nor botany, but to whom a general knowledge of mycology constitutes a useful cultural background.

The book is documented with selected references at the end of each chapter. It is provided with an excellent subject index and a glossary of mycological terms used in the text.

BOOKS RECEIVED

Pharmacology and Therapeutics. 6th ed. By CHARLES SOLOMON and ELIZABETH S. GILL. J. B. Lippincott Co., Philadelphia, 1952. Illustrated. xix + 656 pp. 21 x 14.5 cm. Price $4.50.

Lange's Handbook of Chemistry. 8th ed. Edited by N. A. LANGE and C. M. FORKER. Handbook Publishers, Inc., Sandusky, Ohio. 14 x 20.5 cm. xvi + 1998 pp. Price $7.00.

Maleic Anhydride Derivatives. Reactions of the Double Bond. By LAWRENCE H. FLETT and WILLIAM HOWLETT GARDNER. John Wiley & Sons, Inc., New York; Chapman & Hall, Ltd., London; 1952. x + 269 pp. 15.5 x 23.5 cm. Price $6.50.

Statistical Method in Biological Assay. By D. J. FINNEY. Hafner Publishing Co., New York, 1952. Illustrated. xix + 661 pp. 22 x 15 cm. Price $11.00.

Pharmacology in Clinical Practice. By HARRY BECKMAN. W. B. Saunders Co., Philadelphia, 1952. xx + 839 pp. 17 x 25 cm. Illustrated. Price $12.50.

Standard Values in Blood. ERRETT C. ALBRITTON, Editor. Prepared under the direction of the Committee on the Handbook of Biological Data, American Institute of Biological Sciences, The National Research Council. W. B. Saunders Co., Philadelphia, 1952. x + 199 pp. 21.5 x 28 cm. Price $4.50.

The Literature on Streptomycin: 1944–1952. By SELMAN A. WAKSMAN. Rutgers University Press, New Brunswick, N. J., 1952. xii + 553 pp. 16 x 23.5 cm. Price $5.00.

Encyclopedia of Chemical Technology. Vol. IX. RAYMOND E. KIRK and DONALD F. OTHMER, Editors. Interscience Publishers, Inc., New York, 1952. xvi + 943 pp. 19.5 x 26.5 cm. Illustrated. Price $30.00 (single copy); $25.00 (subscription).

Organic Syntheses. Vol. 32. RICHARD T. ARNOLD, Editor-in-Chief. John Wiley & Sons, Inc., New York; Chapman & Hall, Ltd., London; 1952. vi + 119 pp. 23.5 x 15.5 cm. Price $3.50.

Chemicals of Commerce. By FOSTER DEE SNELL and CORNELIA T. SNELL. D. Van Nostrand Co., Inc., New York, 1952. viii + 587 pp. 22.5 x 14.5 cm. Price $6.50.

Pharmacopoea Danica 1948—Addendum 1952. Udgivet af Farmakopekommissionen. Nyt Nordisk Forlag Arnold Busck, Kobenhavn, 1952. 181 pp. 24 x 16.5 cm.

Quantitative Analysis: Elementary Principles and Practice. By HARVEY DIEHL and G. FREDERICK SMITH. John Wiley & Sons, Inc., New York, 1952. vii + 539 pp. Illustrated. Price $5.00.

Statistics of Bioassay. By C. I. BLISS. Academic Press, Inc., New York, 1952. iii + 628 pp. 15.5 x 23.5 cm. Price $3.50.

Cortone—A Handbook of Therapy. Merck & Co., Inc., Rahway, N. J., 1952. 129 pp. 11 x 17 cm. Distributed free, on request only, to physicians and pharmacists.

Health Resources in the United States. By GEORGE W. BACHMAN and ASSOCIATES. The Brookings Institution, Washington, D. C.; printed by George Banta Publishing Co., Menasha, Wis., 1952. xvi + 344 pp. 15.5 x 23.5 cm. Illustrated. Price $5.00.

Yearbook of the French Chamber of Commerce of the United States, Inc.—1952. 630 Fifth Ave., New York, N. Y. 392 pp. 16 x 24 cm.

Blakiston's Illustrated Pocket Medical Dictionary. NORMAND L. HOERR and ARTHUR OSOL, Editors. The Blakiston Co., New York, 1952. Illustrated. xvi + 1005 pp. + 24 pp. of plates. 12.5 x 18 cm. Price $3.75.

Chemivision. By WILLIAM J. HALE. Destiny Publishers, Haverhill, Mass., 1952. xiii + 207 pp. 13 x 19.5 cm. Price $3.00.

SCORE ON BASES MADE WITH VEEGUM

JUST OFF THE PRESS, Bulletin No. B122 contains the latest technical information on VEEGUM.

VEEGUM is an anhydrous powdered ointment base, easily hydrated in cold water. Viscosity ranges from thin lotions to stiff ointments by simple adjustment of the water content. VEEGUM dispersions are compatible with a wide variety of active ingredients.

VEEGUM combines with other bases to form opaque, washable preparations. It increases the viscosity and suspending ability of polyethylene glycol ointments. VEEGUM blends with petrolatum to give non-tacky, smooth-spreading ointments. Its combined emulsifying and suspending ability make VEEGUM particularly valuable in formulations containing sticky ingredients or those difficult to disperse.

VEEGUM is inorganic, non-toxic, and non-irritating. It is highly purified Colloidal Magnesium Aluminum Silicate.

WRITE TODAY for the complete VEEGUM story.

R. T. VANDERBILT CO.
SPECIALTIES DEPARTMENT
230 Park Avenue (V) New York 17, N. Y.

☐ Please send VEEGUM bulletin No. B122.
☐ Please send sample of VEEGUM.

NAME_____

POSITION_____

(Please attach to, or write on, your company letterhead)

Greater *flexibility* . . . sensitivity . . .
and wider temperature range
with **FISHER** *Unitized*
CONSTANT-TEMPERATURE BATH

A Unitized Bath is a made-to-fit assembly which can be adapted to almost any type of constant-temperature work which can be carried out on a laboratory scale over the range between 20° and 200° C. and within an accuracy of 0.01° C.

The versatility of the Unitized Constant-Temperature Bath is the result of the ease with which different capacity jars, heaters, stirrers, thermoregulators and other necessary units can be combined to serve the exact needs of a particular laboratory requirement.

There are three sizes of bath jars with a capacity ranging to nearly eleven gallons. Close temperature control is assured by a choice of eight electrical heaters and three cooling coils, combined with three sizes of mechanical stirrers. These may be combined with any of four types of thermoregulator and any of Fisher's many different thermometers. Convenient voltage control unit enables user to change the output of any of his heaters. All Unitized Bath Units are easily assembled and interchanged.

A special Unitized Bath assembly is available for the ASTM oil foaming test.

IMMEDIATE DELIVERY FROM STOCK

Write for illustrated booklet which gives complete information and prices on all parts and accessories in this comprehensive line.

Complete stocks of laboratory instruments, apparatus, reagent chemicals, furniture, and supplies at PITTSBURGH, NEW YORK, ST. LOUIS, WASHINGTON, MONTREAL and TORONTO. For more information write: Fisher Scientific Co., 717 Forbes St., Pittsburgh 19, Pennsylvania.

FISHER SCIENTIFIC

America's Largest Manufacturer-Distributor of Laboratory Appliances and Reagent Chemicals